REVIEW ARTICLE

THE CONCEPT OF BIOENHANCERS IN BIOAVAILABILITY ENHANCEMENT OF DRUGS – A PATENT REVIEW

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ABSTRACT:

Maximizing oral bioavailability is therapeutically important because the extent of bioavailability directly influences plasma concentrations and therapeutic efficacy after oral drug administration. Bioavailability of poor bioavailable drugs can be increased from the many available approaches in the literature and the most recent approach is the use of bioavailability enhancers. The use of bioenhancers seems to be a fruitful method for increasing bioavailability of an orally administered drug and nutraceutical. This approach leads to improved formulation with enhanced oral bioavailability of the active ingredient. Bioenhancers constitute an innovative concept and judicious use of bioenhancers lead to reductions in drug cost, toxicity, and other adverse effects, and have a beneficial influence on the national economy. This patent review article classifies and describes the various bioenhancers and emphasizes their diverse mechanism of action by virtue of which they act as bioenhancers.

Key words: Bioenhancer, Bioavailability, Bioefficacy, Synergism, Herbal Drugs, Pgp Inhibitors, CYP3A4 Inhibitors, Solubilizers.
INTRODUCTION:

Today there is a great interest and medical need for the improvement of bioavailability of a large number of drugs which are poorly bioavailable, given for long periods, and are toxic and expensive. Poor bioavailable drugs remain sub-therapeutic because a major portion of a dose never reaches the plasma or exerts its pharmacological effect unless and until very large doses are given which may lead to serious side effects. Any significant improvement in bioavailability will result in lowering the dose or the dose frequency of that particular drug. Incomplete oral bioavailability has various causes. These include poor-dissolution or low aqueous solubility, poor intestinal membrane permeation, degradation of the drug in gastric or intestinal fluids and pre-systemic intestinal or hepatic metabolism [1]. Several approaches have been adopted in the past to maximize oral bioavailability, such as shown in Figure 1.

The present patent review article focuses on the latest revolutionary concept of using the bioenhancers for improving the bioavailability of poor bioavailable drugs, nutraceuticals, metals, and vitamins by various means thereby increasing their bioavailability/bioefficacy.

**Figure 1. Various approaches to maximize oral bioavailability of poor bioavailable drugs**

The use of bioenhancers seems to be a fruitful method for bioenhancement of an orally administered pharmaceutical compound and leads to improved formulation. Based on a traditional system of Indian medicine, bioenhancers constitute an innovative concept and judicious use of them can lead to reductions in drug cost, toxicity, and other adverse effects. Present day research on expensive, toxic and scarce drugs or drugs that exhibit poor bioavailability demands the use of an ideal bioenhancer which should be safe, effective, economical, easily procured, non-addictive etc [2].
A new concept was introduced into science in 1979 with the discovery of piperine as a bioenhancer (piperine still remains the most effective bioenhancer), administration of which significantly increased plasma concentrations of rifampicin, phenytoin, spartein, sulfadiazine, tetracycline, propranolol, and theophylline in humans [5].

Synthetic and naturally occurring toxins and bioaffecting substances as well as recognized pharmaceuticals, such as pro-active, activated and metabolized forms of drugs, drugs consisting of charged, uncharged, hydrophilic, zwitter-ionic, or hydrophobic species can be made bioavailable with the help of bioenhancers with different mechanism of actions but not limited to the following: increasing the penetration or entry of the active drug into the pathogen even where they become persistors, besides inhibiting the capability of pathogens and abnormal tissues to reject the drug. This would eventually ensure the enhanced killing of the pathogenic microorganisms, which are otherwise inaccessible to the active drug, P-gp and CYP 450 have been shown to regulate the oral bioavailability of a majority of drugs, inhibition of both these proteins by the bioenhancers can lead to increased bioavailability of drugs, sensitization of specific receptors like proteins, DNA, RNA etc thus potentiating and prolonging the effect leading to enhanced antibiotic activity aghast pathogens, and disorders and enhancing the absorption and/or inhibiting biotransformation of drugs thereby increasing bioavailability of drugs [4]. The global focus is now on methods aimed at reducing drug dosage, and thus drug treatment cost so that medicines become affordable for wide sections of society, including the financially challenged [2].

What is a bioenhancer?

The rate and extent to which a therapeutically active substance enters systemic circulation and becomes available at the required site of action is termed as bioavailability. Intravenous drugs attain maximum bioavailability, while oral administration yields a reduced percentage due to incomplete drug absorption and first-pass metabolism [3]. A bioenhancer is an agent capable of enhancing bioavailability and bioefficacy of a particular drug with which it is combined. Piperine is the world's first bioavailability enhancer coined and
scientifically validated by Indian scientists at the Regional Research laboratory, Jammu (RRL, now known as Indian Institute of Integrative Medicine) in 1979. Earlier it was extensively used in majority of Ayurvedic formulations as ‘Trikatu’, which is a combination of black pepper (*Piper nigrum*), long pepper (*Piper longum*) and ginger (*Zingiber officinale*) for treatment of a large variety of diseases. The benefits of adding a bioenhancer include reduced drug dosage, reduced cost of the drug, reduced incidence of drug resistance and reduced risk of adverse drug reaction/side effects, reduced requirement of raw material for drug manufacture [4].

**Mechanism of action of bioenhancers**

Generally a bioenhancer may increase the bioavailability of nutraceuticals by acting on gastrointestinal tract to enhance absorption, or by acting on drug metabolism process i.e. inhibiting or reducing the rate of biotransformation of drugs in the liver or intestines, modifying the signaling process between host and pathogen ensuring increased accessibility of the drugs to the pathogens, inhibiting efflux mechanisms frequently encountered with antimalarials, anticancer, antimicrobials etc [5].

**Classification of the bioenhancers**

Bioenhancers can belong to any of the classes based on their mechanism of action by which they increase the bioavailability of other compounds/drugs/nutraceuticals and vitamins etc. A classification of bioenhancers is depicted in Figure 2.

![Classification of Bioenhancers](image)

**Figure 2. Classification of bioenhancers**
I. Solubilizers as bioenhancers

Poor solubility is one of the main causes for poor bioavailability. The fraction of poorly soluble compounds entering clinical development has increased during last decades. Thus, the use of solubilizers as bioenhancers has become a key parameter in optimizing solubility in pharmaceutical research and development [6].

a. Fulvic Acid

Native Shilajit is a blackish-brown exudation containing two classes of organic compounds, namely, (a) humic substances and (b) non-humic organic metabolites. Humic substances have molecular weights ranging from several thousand for humic acids (HAs), to up to several million for polymeric humins (HMs) and only a few hundred for its fulvic acid (FAs) component. The non-humic substances are low molecular weight compounds of marine fossil, plant and microbial origin, occurring in and around shilajit-bearing rocks [7]. Fulvic acid is characterized by having a sponge-like structure punctured by voids of about 200-1000 Å in diameter and a molecular weight, (Mw) of about 700 – 2500. A water-insoluble ingredient can be added to the purified fulvic acid carrier in an amount of about 0.5 to 40% by weight of the fulvic acid carrier filling its voids and this potentiates the bioactivity of a drug, nutritional or cosmetic ingredient. This can be done physical mixing of drug and carrier and/or chemical bonding of drug and carrier like ligand-complex/chelation, reversible covalent bonding and/or charge-transfer complexes etc. Upon dissolution in water, the active ingredient is released to perform its intended active function. Fulvic acid helps augment the bioavailability of drug by enhancing water solubility of active drug ingredients [7] as shown in Table 1.

<table>
<thead>
<tr>
<th>Fulvic acid as bioenhancer</th>
<th>Bioavailability enhancement effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purified Fulvic Acid-Glibenclamide Drug Delivery System</td>
<td>Glibenclamide (Gb) an oral hypoglycaemic agent, sparingly soluble in water, when mixed with purified fulvic acid is completely soluble in water and the composition also exhibits an increased hypoglycaemic action [7].</td>
</tr>
<tr>
<td>Purified Fulvic Acid-Insulin Compositions</td>
<td>Streptozocin (Stz)-induced diabetic (SID) rats were used here. Insulin (I, 1.0 U/kg, p.o.) produced only marginal lowering (&lt;10%) of plasma glucose level (PGL) in SID rats. By contrast, purified fulvic acid-insulin compositions (0.25 U/kg+10 mg/kg, p.o.) and (0.5 U/kg+10 mg/kg, p.o.) produced a 25% and 38% lowering of PGL in SID rates respectively. The effect lasted for 6 hours [7].</td>
</tr>
<tr>
<td>Purified Fulvic Acid-Pentazocin (Ptz) Compositions</td>
<td>Ptz is a narcotic opioid analgetical which shows an extensive first pass effect with oral bioavailability of 30-35%. Ptz-Fulvic acid (5 mg+50 mg) compositions, given</td>
</tr>
</tbody>
</table>
orally, produced a marked analgetic effect which appeared within 30 min and lasted for 180 min [7].

b. Cyclodextrins
Cyclodextrin is known to enhance solubility of a variety of compounds by forming what are called inclusion complexes [8]. Arcari et al.; 1992 demonstrated a dramatic increase in dissolution rate of a silybin and β-cyclodextrin complex (> 90% within 5 minute) compared to non-complexed silybin that is almost insoluble. After administration of the silybin complex in vivo, the silybin concentration in rat bile was nearly 20 times greater than after administration of silybin in its traditional form [9].

c. Gelucires
Gelucires are polyethylene glycol (PEG) glycerides composed of mono-, di- and triglycerides and mono- and diesters of PEG. They are inert semi-solid waxy amphiphilic excipients with surface-active properties that spontaneously form a fine dispersion or emulsion upon contact with water to produce immediate-release solid dosage forms for poorly water-soluble drugs [10]. Attempt has been made in past to improve the peroral bioavailability of silymarin, a herbal hepatoprotectant through the formation of semisolid dispersion system with Gelucire 44/14. Binary systems were prepared by the solvent –fusion method and confirmed by DSC. Increase in solubility of silymarin semisolid dispersion was observed (1.5 to 7.0 folds relative to pure silymarin at 1% to 15% Gelucire 44/14 concentrations) which in turn increased the dissolution rate of silymarin-Gelucire system (91% within 10 minutes). The in-vivo performance was also found to be very good [11]. Examples of solubilizers as bioenhancers of herbal drugs available in patent literature.

Carotenoid compositions of enhanced solubility and bioavailability are described in patent literature. The carotenoid can be β-carotene, lycopene or lutein. The bioenhanced products were made by dry blending and solvent spray drying methods where a mixture comprising of the carotenoid, a solubility-enhancing polymer and a solvent was used and the solvent was removed to produce an amorphous form of the carotenoid. These bioenhanced compositions of carotenoids can be used in pharmaceuticals, nutraceuticals, cosmetic, and personal care products for man and animal [12].

A water-soluble synergistic composition of curcumin, an antioxidant, with a hydrophilic carrier and a fat having enhanced bioavailability is also available in patent literature which is useful for the treatment of depression. The process comprises the steps of dissolving curcumin, antioxidant, a hydrophilic carrier and a fat in a solvent to form a homogenous mass; warming the resultant mass at a temperature ranging from 25°C to 60°C for a period of 4 to 8 hours to obtain a dry wet mass; removing the solvent by evaporation to form dry mass and pulverizing the dry mass to form a fine bioenhanced powder [13].

The solubility and bioavailability of coenzyme Q10 was enhanced with the use of solubility-enhancing polymer by
dry blending and solvent spray drying methods, the steps include a mixture comprising CoQ10, a solubility-enhancing polymer and a solvent and removing the solvent to form amorphous product. The bioenhanced products can be used in pharmaceuticals, nutraceuticals, cosmetic, and personal care products for man and animal [14].

II. P-glycoprotein inhibitors as bioenhancers

The bioenhancers that can increase the bioavailability of an orally administered pharmaceutical compound through the inhibition of P-glycoprotein transport activity in the gut of a mammal are described in this section. These bioenhancers can act as either the inhibitor or the substrate of P-gp in a competitive, uncompetitive, non-competitive manner, mixed or irreversible inhibitor of P-gp drug transport. P-gp inhibitors reduce P-gp active drug transport across the luminal membrane and prevent return of drugs absorbed into the cytoplasm of the enterocytes back to the lumen of the gut. This increases bioavailability by increasing net drug absorption in the gut. The P-gp inhibitors as bioenhancers can be administered with compounds from classes such as aminoquinolines, anilides, anthracycline antibiotics, antiestrogens, benzofurans, cannabinoids, cephalosporines, colchicine, cyclic peptides, epipodophyllotoxins, flavonoids, flavones, imidazole, isoquinolines, macrolides, opioids, phenylalkylamines, phenothiazines, piperazines, piperidines, polyethylene glycols, pyridines, pyridones, pyrimidines, pyrrolidines, quinazolines, quinolines, quinones, rauwolfia alkaloids, retinoids, salicylates, sorbitans, steroids, taxol, triazoles, unsaturated fatty acids, and vinca alkaloids etc [15].

a. Piperine

Piperine, the major plant alkaloid present in *Piper nigrum* Linn (Black pepper) and *Piper longum* Linn (Long pepper), has bioavailability enhancing activity for some nutritional substances and for some drugs [4]. The bioenhancing dose of piperine is approximately 15 mg/person/day and no more than 20 mg/day in divided doses, which corresponds to from several thousands to up to 40,000 times less than the LD50 dose of piperine, as established in various experiments on rodents. The effective bioenhancing dose of piperine for drug compounds varies, but a dose of approximately 10% (w/w) of the active drug could be regarded as an appropriate bioenhancing dose for most drugs [4]. Piperine, or mixtures containing piperine, has been shown to increase the bioavailability, blood levels, and efficacy of many of drugs and nutraceuticals as shown in Figure 3 and Table 2.
Figure 3. Mechanism of actions of piperine as a bioavailability enhancer of drugs and nutraceuticals (Thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione-S-transferase (GST), and glutathione (GSH), Arylhydrocarbon hydroxylation (AHH) and Uridine diphosphate-(UDP) glucuronyltransferase (UGT).

Table 2. Bioavailability enhancement of various drugs and nutraceuticals by piperine

<table>
<thead>
<tr>
<th>Piperine as bioenhancer</th>
<th>Bioavailability enhancement</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piperine and Aflatoxin B1</td>
<td>Piperine enhances bioavailability of aflatoxin B1 in rat tissues. A 10 mg dose of piperine causes a marked increase in serum gonadotropins and a decrease in intratesticular testosterone concentration, despite normal serum testosterone titres in adult male albino rats.</td>
<td>[21]</td>
</tr>
<tr>
<td>Piperine and Phenytoin</td>
<td>Effect of piperine on pharmacokinetics of phenytoin was studied in healthy volunteers. The results of a crossover study, showed that a single daily dose of piperine for 7 days decreased the t1/2α (P &lt; 0.05), prolonged the t1/2 (P &lt; 0.01), and produced a higher AUC (P &lt; 0.05) in comparison to phenytoin alone.</td>
<td>[22]</td>
</tr>
<tr>
<td>Piperine and Pentobarbitone</td>
<td>Effect of piperine on pentobarbitone-induced hypnosis in rats was studied. Piperine treatment in rats, treated chronically with phenobarbitone, significantly potentiated pentobarbitone sleeping time, as compared to the controls. There was no alteration in barbital sodium sleeping time. It is possible that piperine inhibits liver microsomal enzyme system and thereby potentiates the pentobarbitone sleeping</td>
<td>[23]</td>
</tr>
<tr>
<td>Piperine with Propranolol and Theophylline</td>
<td>The effects of piperine on the bioavailability and pharmacokinetics of propranolol and theophylline were studied. An earlier t(\text{max}) and a higher C(\text{max}) and AUC were observed in the subjects who received piperine and propranolol. It produced a higher C(\text{max}), longer t(1/2), and a higher AUC with theophylline.</td>
<td>24</td>
</tr>
<tr>
<td>Piperine and Curcumin</td>
<td>Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers was studied. Piperine, a known inhibitor of hepatic and intestinal glucuronidation, enhanced the serum concentration, extent of absorption, and bioavailability of curcumin in both rats and humans with no adverse effects.</td>
<td>25</td>
</tr>
<tr>
<td>Piperine and β-Carotene</td>
<td>The effectiveness of piperine was evaluated for its ability to improve serum response of β-carotene during oral supplementation using a double-blind, crossover study design. Study suggested that the serum response during oral β-carotene supplementation is improved through the nonspecific, thermogenic property of piperine</td>
<td>26</td>
</tr>
<tr>
<td>Piperine and Coenzyme Q10</td>
<td>In a double-blind study, it is postulated that the bioenhancing mechanism of piperine to increase plasma levels of supplemental coenzyme Q10 is nonspecific and possibly based on its description in the literature as a thermonutrient.</td>
<td>27</td>
</tr>
<tr>
<td>Piperine and Rifampicin</td>
<td>Piperine augments transcription inhibitory activity of rifampicin by several folds against Mycobacterium smegmatis. Combining piperine with rifampicin decreased the dose of rifampicin from 450 to 200 mg.</td>
<td>28</td>
</tr>
<tr>
<td>Piperine and Resveratrol</td>
<td>Effect of piperine on oral bioavailability of resveratrol was studied in mice and the study demonstrated that piperine significantly improves the in vivo bioavailability of resveratrol.</td>
<td>29</td>
</tr>
</tbody>
</table>

Examples of piperine as bioenhancers of drugs and nutraceuticals available in patent literature:
Patent Nos. [30-33] discloses compositions and methods for the improvement of gastrointestinal absorption and systemic utilization of nutrients and nutritional supplements, wherein the compositions comprise a minimum of 98% of pure alkaloid piperine.
Patent Nos. [34-36] discloses tetrahydropiperine, its analogs and derivatives, including dihydropiperine, to enhance the absorption and/or bioavailability of nutrients, drugs and other organic compounds, such as insecticides.
b. Sinomenine

Sinomenine (7, 8-didehydro-4-hydroxy-3, 7-dimethoxy-17-methylmor phinan-6-one) is an alkaloid extracted from Sinomenium acutum Thunb has been used as bioenhancer of Paeoniflorin - a bioactive monoterpene glucoside, which has been widely used to treat inflammation and arthritic conditions. Paeoniflorin has a poor absorption rate and thus a very low bioavailability (3-4%) when administered orally. Co-administration with sinomenine dramatically altered the absorption behaviors of paeoniflorin in rats. The mechanism underlying this improvement of bioavailability of paeoniflorin may be explained by, that sinomenine could decrease the efflux transport of paeoniflorin by P-glycoprotein in the small intestine[38].

c. Genistein

Genistein (5, 7-Dihydroxy-3-(4-hydroxyphenyl) chromen-4-one) known as a phytoestrogen belongs to the isoflavone class of flavonoids. Since genistein was reported to be able to inhibit P-glycoprotein, BCRP and MRP2 efflux function, the intestinal absorption of paclitaxel, a substrate for efflux transports such as P-glycoprotein, BCRP and MRP2 was dramatically increased, co-administered with genistein. In case of the anticancer drug paclitaxel, genistein (10 mg/kg) caused an increase in AUC (54.7%) and a decrease in the total plasma clearance (35.2%) after oral administration of paclitaxel at a dose of 30 mg/kg in rats[39].

III. Inhibitors of cytochrome P450 3A drug biotransformation as bioenhancers

Belonging to a superfamily of hemoproteins which represents the terminal oxidases of the mixed function oxidase system, CYP450 are composed of at least 207 genes that have been named based on the evolutionary relationships of the cytochromes P450. Three CP450 gene families (CYP1, CYP2 and CYP3) appear to be responsible for the metabolism of a large number of structurally diverse drugs. The liver and enterocytes lining the lumen of the intestine contains many isoforms of cytochrome P450 but mostly dominated by a single family of isozymes, 3A, the most important isoforms in drug metabolism[40]. Therefore, this section provides cytochrome P450 3A (CYP3A) inhibitors and enhancers as bioenhancers of drugs and nutraceuticals. Examples of the CYP3A inhibitors include α-naphthoflavone, β-naphthoflavone, apigenin, baicalein, β-myrcene, catechin, 3-phenylpropyl acetate, formononetin, gallic acid, hesperetin, hesperidin, isoquercitrin, lauryl alcohol, luteolin, luteolin-7-glycoside, narigin, nordihydroguaiaretic acid, quercitrin, swertiamarin, terpineol, and trans-cinnamaldehyde. Examples of the CYP3A enhancers include apigenin, formononetin, and luteolin-7-glycoside[40].

Inhibition of CYP3A by bioenhancers in gut epithelia will lead to a total increase in drug bioavailability in the serum. Fewer drug molecules will be metabolized by phase I enzymes in the gut and will not be available for phase II conjugation enzymes. This will lead to increased concentrations of
untransformed drug passing from gut into the blood and onto other tissues in the body [41]. These have the ability to bioenhance the drugs belonging to the classes: acetanilides, anilides, aminoquinolines, benzhydryl compounds, benzodiazepines, benzofurans, cannabinoids, cyclic peptides, dibenzazepines, digitalis gylcosides, ergot alkaloids, flavonoids, imidazoles, quinolines, macrolides, naphthalenes, opiates (or morphinans), oxazines, oxazoles, phenylalkylamines, piperidines, polycyclic aromatic hydrocarbons, pyrrolidines, pyrrolidonones, stilbenes, sulfonyleureas, sulfones, triazoles, tropanes, and vinca alkaloids etc. [42, 43].

a. Naringin

Naringin, a flavonoid glycoside is capable of inhibiting intestinal CYP3A4, CYP3A1, CYP3A2, P-gp and thus acts as a bioenhancer. Pretreatment with oral ingestion of naringin at 3.3 and 10mg/kg improves the AUC for intravenous paclitaxel (40.8% and 49.1%) in a dose dependent manner [44].

b. Quercetin

Quercetin is a dual inhibitor of CYP3A4 and Pgp. It influences the bioavailability of dilitiazem, paclitaxel, digoxin, doxorubicin and tamoxifen and also significantly increased the bioavailability of epigallocatechin gallate, a main anticancer component in green tea with poor bioavailability in rats and humans due to oxidation, metabolism and its efflux [45].

c. Gallic acid esters

Octyl gallate, propyl gallate, lauryl gallate, and methyl gallate are capable of inhibiting the enzyme responsible for drug biotransformation in the gut as a result of which fewer drug molecules will be metabolized by phase I enzymes in the gut and will not be available for phase II conjugation enzymes. This will lead to increased concentrations of untransformed drug passing from the gut into the blood and onto other tissues in the body. At least 1% by weight gallic acid ester relative to the total weight of the formulation, more preferably at least 2%, even more preferably at least 5% is the bioenhancing dose of gallic acid esters. In the presence of the gallic acid ester. Possible mechanisms include competitive, non-competitive, uncompetitive, mixed or irreversible inhibition of CYP3A drug biotransformation. Compounds (or drugs) that can be administered with gallic acid esters includes acetanilides, anilides, aminoquinolines, benzhydryl compounds, benzodiazepines, benzofurans, cannabinoids, cyclic peptides, dibenzazepines, digitalis gylcosides, ergot alkaloids, flavonoids, imidazoles, quinolines, macrolides, naphthalenes, opiates (or morphinans), oxazines, oxazoles, phenylalkylamines, piperidines, polycyclic aromatic hydrocarbons, pyrrolidines, pyrrolidonones, stilbenes, sulfonyleureas, sulfones, triazoles, tropanes, and vinca alkaloids etc [46].

Sudipta Basu et al. 2012 investigated the potential pharmacokinetic interactions of rosuvastatin (RSV) with herbal bioenhancers (such as piperine, gallic acid and cinnamic acid) in healthy male C57 BL/6 mice. After per oral (p.o.) co-administered of RSV (5 mg/kg) with piperine, gallic acid and cinnamic acid (5 mg/kg) intravenous exposure (AUC_{last}) significantly increased by 174.51%, 176.65% and 181.08%
respectively than alone group (control). Compared with the control (alone) group, p.o. co-administered of piperine, gallic acid and cinnamic acid (5 mg/kg) significantly increased the oral exposure (AUC\text{last}) of RSV (25 mg/kg) by 1.35-fold, 1.66-fold and 1.62-fold respectively. The absolute oral bioavailability of rosuvastatin alone increased from 33 % upto 45, 56 and 54% with rosuvastatin co-administered with per oral dose of piperine, gallic acid and cinnamic acid respectively showing 15, 19 and 18% increase in oral availability of rosuvastatin when co-administered with piperine, gallic acid and cinnamic acid respectively in C57BL/6 male mice [47].

IV. BCRP inhibitors as bioenhancer

Breast Cancer Resistance Protein (BCRP) is a protein overexpressed in breast carcinoma cell lines. The oral administration of BCRP inhibitors together with pharmaceutical compounds enhances the bioavailability of the latter. Thus, bioenhancer being an inhibitor of BCRP mediated or related transport for enhancing the bioavailability of an orally administered drug in tumor cells, could be a useful target to approach with a view to increasing oral bioavailability of drugs [48].

The BCRP inhibitor inhibits drug back flux from the blood or epithelial lumen. Thus, the return of drugs absorbed into the cytoplasm of enterocytes back to the lumen of the gut is inhibited. Preferably the bioenhancer, i.e. the inhibitor of BCRP, will bind BCRP quickly and inhibit while the drug is passing through the enterocyte. The inhibitor can be reversible or irreversible. If it is reversible, the bioenhancer will pass through the liver and be removed. The bioenhancer can act as a competitive, non-competitive, mixed or an irreversible inhibitor. Suitably, a bioenhancer can be selected from substances that are related to known substrates for BCRP such as acridine, quinoline, isoquinoline, indolizino-quinoline, camptothecin, anthraquinone, quinazoline, bisanthrene and rhodamine. Alternatively, a bioenhancer can be selected from vinca alkaloids, fatty acids, triazoles, taxol and derivatives thereof, pyrroliodines, piperazines, pipеридines, pyridines, pyridones, pyrroliodines, retinoids, salicylates, sorbitans, phenothiazines, polyethylene glycols, colchicine, cephalosporines, cannabiods, cyclic peptides, flavones, flavenoids, opioids, phenylalkylamines, aminoacridines, aminoquinolines, anilides, anthracyclines, antibiotics, antiestrogens, imidazoles, (iso)quinolines, benzo[urans, benzodiazepines, benzhydryl compounds, benzazepines, dibenzazepines, epipodophyllotoxins, macrolides, rauwolfia alkaloids, and steroids etc [48].

a. Camptothecin

The use of camptothecin as inhibitor of BCRP is discussed in details in patent literature [49-53]. Patent No. [49] discloses that co-administration of a single oral dose of derivative of camptothecin (GF120918) resulted in a profoundly increased systemic exposure to oral topotecan. The increase in the area under the curve (AUC) was approximately 6 folds. Patent No. [54] discloses a method for increasing the systemic exposure of cells selected from tumor cells and normal cells to an orally administered pharmaceutically active compound, wherein a bioenhancer comprising an inhibitor of BCRP is
orally administered concomitantly with said orally administered pharmaceutically active compound for increased bioavailability.

V. Permeability enhancers as bioenhancers

Essential oils are used as bioavailability enhancers and they reduces, inter- and intra-individual variability of an orally administered hydrophobic pharmaceutical compound by co-administering the pharmaceutical compound with an essential oil or a component of essential oil in an amount sufficient to improve the bioavailability [55-57].

a. Carum carvi

Carum carvi is a prized culinary herb used extensively in India for flavouring of food and medicinally it is an effective gastric stimulant, carminative and anthelmintic [58]. The extracts of Carum carvi are used as bioenhancers, either alone or in combination with piperine or Zingiber officinale extract to improve the bioavailability of a wide variety of drugs for example, antibiotics, antifungals, anti-virals, anticancer, cardiovascular, CNS, anti-inflammatory/anti-arhritic, anti-TB/anti-leprosy, anti-histaminic/respiratory disorders, corticosteroids, immunosuppressants, anti-ulcer etc [59]. The mechanism of action of Carum carvi as a permeability enhancer/bioenhancer is attributable to one or more than one of the following reasons: (a) Promoting the absorption of drugs from GIT, (b) Inhibiting or reducing the rate of biotransformation of drugs in the liver or intestines, (c) Modifying the immune system in a way that the overall requirement of the drug is reduced substantially, (d) Increasing the penetration or the entry into the pathogens even where they become persisters within the macrophages such as for Mycobacterium tuberculosis and such others. The effective dose of the bioenhancer extract used is in the range of 5 to 100 mg/kg body weight in which the dose fraction of Carum carvi used ranges from 1 to 55 mg, Zingiber officinale (10-150 mg/kg body weight) and piperine (3-15 mg/kg body weight). Together they have increased significantly (25-300%), the bioavailability of a number of classes of drugs [60,61].

b. Cuminum cyminium

The essential oil from Cuminum cyminium is being used as bioenhancer and a lot of information is available in patent literature [62-66]. Patent No. [67] relates to the use of Cuminum cyminium as bioenhancer to decrease the resistance of microbial strains to anti-infective such as antibiotics and antifungals by potentiating the activities of anti-infective. This may be useful to reduce resistance in bacteria and yeast to aid in the treatment of certain infections and diseases and to lower the concentration of anti-infectives necessary to inhibit the growth of microbial strains. Patent No. [68] discloses a bioavailability-facilitating composition comprising of an effective amount of an extract and/or at least one bioactive fraction from Cuminum cyminum; one or more additive selected from drugs, nutrients, vitamins, nutraceuticals, herbal drugs/products, micro nutrients, antioxidants along with pharmaceutically acceptable additives / excipient, and- optionally, an effective amount of piperine or extract/fraction of
piper nigrum or piper longum for increased bioavailability.

c. Ginger (Zingiber officinale)

Ginger is the dried underground stem or rhizome of the zingiberous, herbaceous plant Zingiber officinale Linn, which constitutes one of the most important major spices of India. Traditionally it is used as a carminative and stimulant to the gastro-intestinal tract. It is much in vogue as a household remedy for flatulence and colic. Externally, ginger is used as a local stimulant and rubefacient. Ginger oleoresin (Gingerin) generally contains following types of compounds: Gingerols, Zingerones, Shogaols, volatile oil, resins, phenols etc. The oil contains sesquiterpene hydrocarbons (50% or more), sesquiterpene alcohols, monoterpenoids and associated compounds, esters of acetic acid and caprylic acid and a trace of chavicol. The role of ginger is to regulate intestinal function to facilitate absorption [69]. Patent No. [69] discloses a bioenhancing composition containing extract from the plant Zingiber officinale in combination with drugs, nutrients, nutraceuticals, micronutrients and herbal drugs/products and optionally containing piperine as a bioavailability enhancer and this increased significantly (25-435%), the bioavailability of a number of classes of drugs belonging to antibiotics like Azithromycin (85%), Erythromycin (105%), Cephalexin (85%), Cefadroxil (65%), Amoxycillin (90%) and Cloxacillin (90%), antifungals, antivirals, anticancer, cardiovascular, CNS, anti-inflammatory/anti-arthritic, anti-TB/antileprosy, anti-histaminic/respiratory disorders, corticosteroids, immunospressants, anti-ulcer. The amount of Zingiber officinale extract used is in the range of about 2.0 to 250 mg. The amount of Zingiber officinale fraction/pure isolates used is in the range of about 0.5 to 75 mg and the amount of piperine used is in the range of about 5 to 50 mg. Also, Patent No. [70] discloses a composition to enhance the bioavailability of curcumin with vanilla and ginger, wherein the extracts of ginger and vanilla are rich in gingerol and vanillin respectively.

d. Garlic (Allium sativum)

Allicin, the phytomolecule in garlic (Allium sativum) enhances the activity of amphotericin B against yeast (Sacchromyces cerevisiae) and fungal (Candida albicans, Aspergillus fumigatus) infection. Although allicin was ineffective in promoting AmB-induced plasma membrane disability, this compound enhanced amphotericin-induced structural damage to the vacuolar membrane even at a non-lethal dose of the antibiotic [71].

VI. Miscellaneous

a. Lysergol

Lysergol (9, 10-Didehydro-6-methylergoline-8-α-methanol) is a promising herbal bioenhancer phytomolecule obtained from morning glory plant (Ipomoea spp.) which enhances the killing activities of different antibiotics on bacteria. It has been isolated from higher plants like Rivea corymbosa, Ipomoea violacea, and Ipomoea muricata. The seeds of Ipomoea muricata are a good source of clavine alkaloids (0.49% of total alkaloid, out of which lysergol
constitutes 53% and chanoclavine 37% [72].

Patil et al. 2012 reported that lysergol improved the bioavailability of berberine after oral administration in Sprague-Dawley rats. The plasma concentration of berberine rose from a maximum of 112 ng/mL in the absence of lysergol to 191 ng/mL in its presence, an increase of 70%. So, lysergol had a marked effect on the bioavailability of berberine, although the absorption and elimination characteristics of berberine were unchanged. The bioenhancement might be by inhibiting the metabolism of berberine or modifying its transport across cell membranes [73].

In Patent No 74 it is disclosed that lysergol enhanced the antimicrobial effect of the antibiotic compounds like rifampicin, tetracycline, and ampicillin in the range of 2–12 folds by reducing the dosage of antibiotics while increasing the efficiency of absorption of nutritional elements. The effective amount of lysergol as a bioenhancer was in the range of 1–10 µg/mL but preferably 10µg/mL. Lysergol is effective against broad spectrum microbes, Gram-positive and Gram-negative, consisting E. coli (ATCC 10536), B. subtilis (ATCC 6051), M. smegmatis (ATCC 14468), and other similar microbes.

c. Cow urine distillate

An effective amount of bioactive fraction from cow urine distillate is the only bioavailability facilitator obtained from animal source and is pharmaceutically acceptable bioenhancer. It has antitoxic activity against cadmium chloride toxicity and it can be used as bioenhancer of zinc. In a study, fertility rate decreased to 0% when male mice were exposed to cadmium chloride. However fertility rate increased up to 90% along with viability when animals were exposed to cadmium chloride – cow urine distillate and zinc sulphate 77. It is being used as bioenhancer for anticancer compounds, antibiotics, drugs, therapeutic and nutraceutic agents, ions and similar molecules which are targeted to the living systems 78. Cow urine distillate is more effective as a bioenhancer than cow urine. It facilitates the absorption of drugs across cell membrane. Transport of antibiotics like rifampicin, tetracycline and ampicillin across the gut wall up to 2-7 folds has been achieved.
with it [79]. Patent No. [80] disclose a pharmaceutical composition comprising an antibiotic and cow urine distillate in an amount effective to enhance antimicrobial effect of the antibiotic. The antibiotic can be an antifungal agent like quinolone, fluoroquinolone, azoles, clotrimazole, mystatin or amphotericin [81, 82].

d. Glycyrrhiza glabra

Active component of liquorice responsible for its bioenhancing activity is Glycyrrhizin - a non-alkaloid compound which enhances the bioavailability of antibiotics and other drugs including anti-infective and anticancer agents. The molecule facilitates the absorption/uptake of antibiotics and other molecules across the cell membrane in plant and animal cells as well as Gram-positive and Gram-negative bacteria. It has no antimicrobial or cytotoxic activity of its own, is a safe candidate to reduce the drug dosage towards circumventing the problem of drug resistance and the other side effects in anti-infective and anti-cancer therapies. The bioenhancing concentration of glycyrrhizin ranges from 0.05 to 50% of the weight of the antibacterial compounds, 0.10 to 10% of the weight of the nutraceutical compounds and 0.25 to 20% of the weight of the antifungal agents [83]. It enhances cell division inhibitory activity of anticancerous drug ‘Taxol’ (paclitxel®) by 5 folds against the growth and multiplication of breast cancer cell line MCF-7. Glycyrrhizin is also reported to enhance (2-6 fold) transport of antibiotics like rifampicin, tetracycline, nalidixic acid, ampicillin and vitamins B1 and B12 across the gut membrane [84]. The Patent No. [85] provides use of the extract or the compound obtained from the plant Glycyrrhiza glabra, as a bioenhancer and bioavailability and facilitator of nutritional compounds and drugs and molecules selected from anti-infective and anti-cancer agents.

RESEARCH AGENDA WITH HERBAL DRUGS – POOR BIOAVAILABILITY ISSUES AND SCOPE FOR BIOENHANCERS:

There is a huge demand for both experimental and clinical research to validate the potential of herbals, especially from industry-independent sources, since industry will likely not support necessary research activities due to (seemingly) low profit expectations [86]. Sound basic research on and controlled clinical studies with certain herbals and especially their active ingredients in chronic diseases is important, since novel anticancer, antifibrotic and anti-inflammatory activities will likely be discovered. From the studies and clinical trials conducted till date on herbal drugs, most important agenda is that the herbal drugs suffer from poor bioavailability issues within the body like low intrinsic activity, poor absorption, high rate of metabolism, inactivity of metabolic products and/or rapid elimination and clearance from the body. Therefore, studies to date have suggested a strong intrinsic activity and, hence, efficacy of certain herbal drugs like curcumin, berberine hydrochloride, silymarin, resveratol, quercetin as therapeutic agents for various ailments and simultaneously, problems related to absorption, distribution, metabolism and excretion of these like poor solubility, poor absorption, low serum levels, short half-life, limited tissue distribution and
rapid metabolism that severely curtail bioavailability of these herbal drugs have come into limelight.

Today curcumin has been widely acknowledged globally as a "wonder drug of the future" because of its great potential abilities to prevent and treat a wide spectrum of chronic diseases by exhibiting anti-inflammatory, antioxidant, antimicrobial, and anticarcinogenic activities, hepato and nephro-protective, thrombosis suppressing, myocardial infarction protective, hypoglycemic and antirheumatic effects. In addition, it has been proved to be remarkably safe in animal studies and in phase I clinical trials even at high doses (up to 12 g/day). However, the major problem limiting the exploitation of its potentially valuable therapeutic effects is its low bioavailability [18].

Berberine hydrochloride is a conventional component in Chinese medicine, and is characterized by a diversity of pharmacological effects. However, due to its hydrophobic properties, along with poor stability and bioavailability, the application of berberine hydrochloride was hampered for a long time. In recent years, the pharmaceutical preparation of berberine hydrochloride has improved to achieve good prospects for clinical application, especially for novel nanoparticulate delivery systems. Moreover, anticancer activity and novel mechanisms have been explored, the chance of regulating glucose and lipid metabolism in cancer cells showing more potential than ever. Therefore, it is expected that appropriate pharmaceutical procedures could be applied to the enormous potential for anticancer efficacy, to give some new insights into anticancer drug preparation in Chinese medicine [87].

Evaporative precipitation of nanosuspension (EPN) was used to fabricate nanoparticles of a poorly water-soluble antimalarial drug, artemisinin (ART), with the aim of enhancing its dissolution rate [88].

Genistein has been shown to possess anticancer activities in different experimental systems, yet the same effects could not be translated in the clinical setting due to its poor bioavailability. Newer formulations of genistein such as diindolylmethane (BDIM) from Bioreseponse Inc. has shown some enhanced bioavailability [89].

Resveratrol (3, 5, 4'-trihydroxy-trans-stilbene) is a phytoalexin produced naturally by several plants when under attack by pathogens such as bacteria or fungi. Resveratrol and its effects is currently a topic of numerous animal and human studies. In mouse and rat experiments, anti-cancer, anti-inflammatory, blood-sugar-lowering and other beneficial cardiovascular effects of resveratrol have been reported. However, most of these results have yet to be replicated in humans. As with other natural chemopreventive agents, resveratrol also has a very short half life and is rapidly glucoronated and sulfonated, aiding its rapid turnover and excretion. Therefore, researchers focused on ways to enhance the bioavailability of resveratrol by different approaches and nano based studies were among the major driving force in this area. The earliest reported nano formulation of resveratrol comes from a study, where they prepared resveratrol
chitosan nanoparticles with free amine groups on the surface so as to conjugate ligands, which will actively target to special tissues or organs [90].

Research in proper direction is required in order to increase the bioavailability of these herbal drugs and use of bioenhancer with appropriate mechanism of action is needed.

**Future bioenhancers**

Discussed below are few compounds which have the potential to increase the bioavailability of certain drugs and nutraceuticals. More studies will have to be carried out to unearth the mechanism and establish their role as a promising bioenhancer. These are Aloe vera, Morin, Noni, Hesperidin, Capsicin, Capmul and Cinnamic acid etc. Capmul 708G EP is a glycerin monoester of caprylic acid produced from edible fats and oils. It acts as a solubilizer, carrier (vehicle), bioenhancer, emulsifier / co-emulsifier, penetration enhancer (dermal) etc [91]. A lot has been published in the form of review articles on the role of bioenhancers for increasing the bioavailability of poorly bioavailable drugs. Many herbal compounds have been classified as bioenhancers owing to their bioenhancing effects for other drugs [92-95].

**Regulatory aspects and hurdles in the use of bioenhancers**

The hurdles in the use of bioenhancers are large scale production of bioenhancer is not an easy task. Phytomolecules as bioenhancers are extracted in meager amounts from natural sources and this imposes a big hurdle in their use. From the commercialization point of view large scale use of bioenhancers is needed rather than laboratory scale. Secondly, it is important to get regulatory approval for them. Detailed study of their physicochemical and pharmacokinetic properties is required to ensure their safety profile. And lastly, without sufficient clinical studies they cannot be incorporated into formulations and marketed directly for public usage [96]. The bioenhancing effect of phytochemicals as natural bioenhancers of wide variety of drugs and nutraceuticals in animals and humans need a lot of experimentation of experimental animals. Lack of information on the mechanism of action, adverse effects, evaluation of toxicity indices of the extracts have to be determined. Research should be focused on all these parameters of safety, compatibility with drugs and nutraceuticals, toxicity, efficacy and mechanism of action of these bioenhancers. Finally optimization of pharmacokinetics of these bioenhancers is required to establish them as effective bioenhancers.

**Regulatory approved formulation with bioenhancers**

Drug Control General of India (DCGI) has approved the marketing of antitubercular formulation named Risorine in the Indian market, containing 200 mg of rifampicin, 300 mg of isoniazid (INH) and 10 mg of Piperine by Cadila Pharma in November 2009. Piperine increased bioavailability of rifampicin by about 60%. Therefore, adding the bioenhancer ‘Piperine’ reduced the dose of rifampicin from 450to 200 mg. Thus, reducing the
dosage, cost and toxicity of rifampicin [97].

CONCLUSION:

Formulations containing natural bioenhancers with enhanced bioavailability and efficacy of active ingredients opens up new horizon in pharmaceutical and healthcare sector. Reduced dose and cost along with safety and efficacy is the unique selling point of such formulations. Today a lot of research is going on various classes of bioactives for their bioenhancing ability so that more and more, better pharmaceutical formulations come in the markets.

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CONFLICT OF INTEREST:

Authors declare no conflict of interest.

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ABBREVIATIONS:

AB: Absolute bioavailability
AUC: Area under the plasma concentration-time curve
AUClast: Area under the plasma concentration-time curve from 0 hours to the last measurable concentration
BCRP: Breast cancer resistant protein
CYP450: Cytochrome P450
Cmax: Peak concentration
DSC: Differential scanning calorimetry
DNA: Deoxy ribonucleic acid
FA: Fulvic acid
LD50: Half-maximal lethal dose
Mw: Molecular weight
MRT: Mean residence time
MRP: Multidrug resistance associated protein
MCF-7: Michigan cancer foundation -7
p.o: Per oral
Pgp: P glycoprotein
QSAR: Quantitative structure activity relationship
RNA: Ribonucleic acid
t1/2: Terminal halflife
T1/2: Absorption half life
tmax: Time to reach peak concentration


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