Recent Advances on the Role of Basic Sciences in Ayurvedic Medicine

Chief Editor
Ratnesh Kumar Rao

Editor
Prof. J.S. Tripathi

Published by
Mahima Research Foundation and Social Welfare
194, Karaundi, Banaras Hindu University, Varanasi-221005, UP, India
Reg. # 643/2007-2008

Published by

©Mahima Research Foundation and Social Welfare

194, Karaundi, Banaras Hindu University, Varanasi-221005, UP, India

Reg. # 643/2007-2008

ISBN: 978-81-926935-3-8

Year – 2014

Price: Rs. 500.00

$ 100.00

Typing, Formatting and Designed by :

Mahima Research Foundation and Social Welfare

Printed by

Mahima Publications

194, Karaundi, Banaras Hindu University, Varanasi-221005

Editorial Board

Year – 2014

Published by
©Mahima Research Foundation and Social Welfare
194, Karaundi, Banaras Hindu University, Varanasi-221005
Reg. # 643/2007-2008

Printed by
Mahima Publications
194, Karaundi, Banaras Hindu University, Varanasi-221005

AN INTEGRATED APPROACH TO THE BIOAVAILABILITY ENHANCERS

Satyapal Singh[1], J.S. Tripathi[2] and N.P. Rai[3]

[1]Senior Resident & Research Scholar, department of Kayachikitsa, faculty of Ayurveda, Institute of Medical Sciences ,Banaras Hindu University , Varanasi, Uttar Pradesh, India, Mob: 09450035793, E-mail:spmairti@gmail.com, [2]Professor, department of Kayachikitsa, faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India and [3]Professor & Head, department of Kayachikitsa, faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India.

Bioavailability is defined as the rate and extent (amount) of absorption of orally administered unchanged drug from its dosage form that reaches the systemic circulation. It is one of the important parameter to achieve desired concentration of drug in systemic circulation for pharmacological response to be shown. Many studies have clearly shown that diets rich in plant foods protect humans against various diseases such as cancer, neurodegenerative disorder and cardiovascular diseases [1]. Plant foods contain a variety of medicinally active constituents such as alkaloids (piperine) and polyphenols (gallic acid and cinnamic acid). Polyphenols are abundant micronutrients in our diet, present in many fruits and vegetables [2] and evidence shows their role in the prevention of many diseases [3]. Therefore, these are increasingly regarded as herbal medicines. [4].

Bioavailability differs greatly from one polyphenol to another [5]. Gallic acid (GA), cinnamic acid (CA) and isoflavones are the well-absorbed polyphenols, followed by catechins, flavanones, and quercetin glucosides, but with different kinetics [6,7]. For a drug administered orally, the 3 main reasons for its decreased bioavailability are-1. Decreased absorption. 2. Destabilisation or destruction of drug. 3. First –pass/presystemic metabolism.

Bioavailability enhancers of natural origin are phytomolecules based on ancient knowledge of Ayurveda. They, reduce the dose, shorten the treatment period, thus reducing drug-resistance problems. The treatment is made cost effective, minimizing drug toxicity and adverse reactions. When used in combination with number of drug classes such as antibiotics, anti-tuberculosis, antiviral, antifungal and anti-cancerous drugs they are quite effective.

Bioavailability and absorption enhancement through co-administration of drugs with naturally occurring compounds from plants are considered to be very simple and relatively safe. They increase the bioavailability and absorption of the co-administered drugs. Uses of bio-enhancers are also applicable in veterinary practice since bioavailability of drugs and nutrients is of equal relevance to animals as to humans [8].

Bioavailability Enhancers: About 60 % of the world population use plant based medicines and about one third of the world countries depend on herbal medicines. A bio-enhancer is an agent which is used to enhance the

bioavailability and efficacy of a drug with which it is co-administered, without any pharmacological activity of its own at the therapeutic dose[9].

Bio-enhancers are drug fascilitator, they have the tendency to decrease the dose of active drug. They are molecules which by themselves do not show typical drug activity, but when used in combination enhance the activity of drug molecule in several ways including increasing bioavailability of the drug across the membrane, potentiating the drug molecule by conformational interaction, acting as receptor for drug molecule and making target cells more receptive to drugs. Many allopathic drugs have suffered with the low bioavailability upon oral administration due to poor permeation across the gastrointestinal epithelia.

Historical Aspect: The concept of bio-enhancers or bio-potentiators is new to the modern science, but many drugs as bioavailability enhancers used in Ayurveda since time immemorial. Therefore, basically this concept originated in Ayurveda. It was first time reported by Bose in 1929, who described the increase in the anti-asthmatic effects of vasaka (Adhatoda vasica) leaves by the addition of long pepper to it. The term bioavailability enhancer was first coined by Indian Scientists at the Regional Research laboratory, Jammu (RRL, now known as Indian Institute of Integrative Medicine) , who discovered and scientifically validated piperine as the world's first bioavailability enhancer in 1979. The concept of bio-enhancers of herbal origin can be tracked back from the ancient knowledge of Ayurvedic system of medicine. Ayurvedic texts have been mentioned a preparation "Trikatu", meaning three acrids. It refers to a combination of black pepper (Piper nigrum Linn.), long pepper (Piper longum Linn.), and ginger (Zingiber officinale Rosc.), which contains active component piperine, which enhances the bioavailability of drugs, nutrients, and vitamins. Modified from[10].

Objectives of Bioavailability Studies

- Primary stages of development of a suitable dosage form for a new drug entity to obtain evidence of its therapeutic utility.
- Determination of influence of excipients, patient related factors and possible interaction with other drugs on the efficiency of absorption.
- Development of new formulations of the existing drugs.
- Comparision of availability of a drug substance from different dosage forms or from the same dosage form produced by different manufacturers.

Need for Bio-enhancers: The presence of intestinal barriers produces a major challenge for drug delivery. Various physiological factors that reduce the oral bioavailability of drugs include disease state, gastric emptying rate, circadian differences, interaction with food, intestinal motility and presence of intestinal microflora. Apart from these, many pharmaceutical barriers including poor solubility in gastrointestinal pH, high molecular weight of drugs make the oral delivery quiet exigent one. There are many structures in the intestinal epithelium which serve as barriers to the transfer of drugs from the gastrointestinal track to the systemic circulation. An aqueous stagnant

layer due to its hydrophilic nature is potential barrier to the absorption of drugs.

A drug with poor bioavailability is one with poor aqueous solubility, slow dissolution rate in biological fluids, poor stability of dissolved drug at physiological pH, poor permeation through biomembrane, extensive presystemic metabolism. Poorly water soluble drugs often require high doses in order to reach therapeutic plasma concentrations after oral administration. Low aqueous solubility is the major problem encountered with formulation development of new chemical entities. Any drug to be absorbed must be present in the form of an aqueous solution at the site of absorption.

Poor solubility remains a major challenge for pharmaceutical industry, which is now considered to be an area of prime importance in the field of biomedical research. Approximately 40% new molecular entities (NMEs) synthesized in pharmaceutical R&Ds with advanced combinatorial chemistry and computer aided drug designing (CADD) approaches, suffer from poor solubility and bioavailability related issues[11].

Drug Absorption Barriers: The drug must cross the epithelial barrier of the intestinal mucosa for it to be transported from the lumen of the gut into the systemic circulation and exert its biological actions. There are many anatomical and biological barriers for the oral drug delivery system to penetrate the epithelial membrane. There are many structures in the intestinal epithelium which serve as barriers to the transfer of drugs from the gastrointestinal tract to the systemic circulation. An aqueous stagnant layer due to its hydrophilic nature is potential barrier to the absorption of drugs. The membranes around cells are lipid bilayers containing proteins such as receptors and carrier molecules. Drugs cross the lipid membrane by passive diffusion or carrier-mediated transport which involves the spending of energy. For the passage of small water-soluble molecules such as ethanol there are aqueous channels within the proteins. The drug molecules larger than about 0.4 nm face difficulty in passing through these aqueous channels[2].

Routes of Drug Transport through Intestinal Epithelial Cells: 1. Passive paracellular transport pathway. 2. Passive transcellular transport pathways. 3. Carrier mediated efflux transport pathway. 4. Carrier mediated active transport (transcytosis) pathways.

Table.1. Showing overview of various transporters found in the intestinal tract affecting oral absorption of drugs.[12]

S N	Transporter	Location	Role in drug transport
1	ABCB1/MDR1/P-gp	High amount in the lining of small intestine	Acts as a rate-limiting barrier for oral absorption of drugs and creates multidrug resistance to chemotherapy as with antibiotics and anticancer drugs and Acts as a substrate for transportation of drugs like digoxin, erythromycin & atorvastatin, shows competitive inhibition upon co-administration.
2	ABCC2/MRP2	Highly expressed in small intestine as compared to MDR1	Limit the absorption of food-derived carcinogen.
3	ABCG2/BCRP	Considerable amount in intestinal epithelial villous cells	Blocks the absorption of tropotecan.

4	OAT	Abundantly found in apical membrane of intestinal enterocyte cells	Restricts the absorption of drugs present in ionized state in intestine.
5	OATB	Expressed at the apical membrane of human enterocytes	pH dependent transport of anionic drugs.
6	OCT	Surface of apical and basolateral membrane	Transportation of amino acid L-carnitine, steroidal hormones, bile salts, bilirubin, benzyl penicillin, and digoxin.

ABCB1-ATP binding cassette transporters subfamily B1; MDR1-Multidrug resistance protein 1; ABCC2-ATP binding cassette transporters subfamily C2; MRP2-Multidrug resis-tance associated protein 2; ABCG2- ATP binding cassette transporters subfamily G2; BCRP-Breast cancer resistant protein; OAT-Organic anion transporter; OATB-Organic aniontransporter subfamily B; OCT-Organic carnitine transporter.

Classification of Bio-enhancers: Bio-enhancers are broadly classified[13, 14] depending on two parameters

- Based on origin: it is further classified into two categories, plant origin and animal origin.
  1. Plant origin: Stevia, Piperine, Capsaicin, Allicin, Ginger, etc.
  2. Animal origin: Cow urine distillate.
- Based on Mechanism of Action: these are further categorize into following three…
  1. Inhibitors of P-gp efflux pumps & other efflux pumps: Carum carvi (caraway), Genistein, Sinomenine, cuminum cyminum (black cumin), Naringin, Quercetin.
  2. Suppressors of Cyp -450 enzyme and its isoenzymes: Naringin, Gallic acid, Quercetin.
  3. Regulators of GIT function to facilitate better absorption: Aloes, Niaziridin (drumstick pods), Ginger, glycyrrhizin (Liquorice).

Novel Properties of Bio-enhancers

- Nontoxic to humans or animals.
- Should be effective at a very low concentration in a combination.
- Should be easy to formulate.
- To enhance uptake/absorption and activity of the drug molecules.
- Should not possess any pharmacological activity of its own at the therapeutic dose used. [15]

Methods for Enhancing Bioavailability: Methods of increasing bioavailability of a drug correspondingly increase the level of drug in the bloodstream, and thus the efficacy, which in turn reduces the drug dosage required to achieve a given therapeutic effect. Following four methods has been used to enhance the bioavailability of drugs.

Absorption Enhancers: Many of the absorption enhancers are effective in improving the intestinal absorption, such as bile salts, surfactants, fatty acids, chelating agents, salicylates and polymers.[16, 17] Surfactants including bile, bile salts and fatty acids act as absorption enhancers by increasing the solubility of hydrophobic drugs in the aqueous layer or by increasing the fluidity of the apical and basolateral membranes. Calcium chelators such

as EGTA and EDTA reduce the extracellular calcium concentration, leading to the disruption of cell-cell contacts. Chitosan, particularly trimethylated chitosan, was reported to increase drug absorption via paracellular route by redistribution of the cytoskeletal F-actin, causing the opening of the tight junctions. Modified from[2].

Table. 2. Summarizes the types of various absorption enhancers on the basis of their chemical properties. Modified from[2].

S.N.	Category	Example
1	Bile salts	Sodium cholate , Sodium deoxycholate
2	Non-ionic surfactants	Polyoxyethylene alkyl ethers ,Polyoxyethylene alkyl esters & Polysorbates
3	Ionic surfactants	Sodium lauryl sulfate & Dioctyl sodium sulfosuccinate
4	Fatty acids	Sodium caprate , Oleic acid , Glycerides ,Natural oils Medium-chain glycerides, Phospholipids, Polyoxyethylene glyceryl esters, Acyl carnitines and cholines , Palmitoyl carnitine & Lauroyl choline
5	Salicylates	Sodium salicylate , Sodium methoxysalicylate
6	Chelating agents	EGTA , EDTA
7	Swellable polymers	Starch , Polycarbophil & Chitosan
8	Others	Citric acid

Prodrugs: Throughout the past decades prodrug approaches have been used to improve the pharmaceutical properties such as solubility, taste, odor, stability etc. Designing of prodrug is also used to improve the physicochemical properties such as compound lipophilicity and solubility to prevail over the pharmacokinetic demerits associated with drug molecules. The chemical decomposition and presystemic metabolism is reduced by the use of prodrug approach. To enhance the drug absorption and bioavailability, chemical modification of drugs to produce prodrugs and more permeable analogues has been widely studied as a useful approach. Various ampicillin derivatives are one of the well-known examples of increasing the lipophilicity of agents to enhance absorption of a polar drug by prodrug strategy. Ampicillin due to its hydrophilic nature is only 30-40% absorbed from the gastrointestinal tract. By esterification of carboxyl group of ampicillin the prodrugs of ampicillin such as pivampicilline, bacampicilln and talampicillin were synthesized. These prodrugs were more lipophilic than the parent compound following oral administration and they showed higher bioavailability in comparison with ampicillin[10].

P-glycoprotein Inhibitors: The application of P-gp inhibitors in improving peroral drug delivery has gained special interest. Several studies to enhance oral bioavailability have demonstrated the possible use of P-gp inhibitors that reverse P-gp-mediated efflux in an attempt to improve the efficiency of drug transport across the epithelia. P-gp inhibitors influences metabolism, absorption, distribution and elimination of P-gp substrates in the process of modulating pharmacokinetics [18].

Dosage Form and Other Pharmaceutical Approaches: Besides chemical modification approaches, utilization of permeability-enhancing dosage forms is one of the most practical approaches to improve the intestinal absorption of poorly absorbed drugs. Various dosage formulations such as liposomes and

emulsions enhanced the intestinal absorption of insoluble drugs. Particle size reduction such as micronization, nanoparticular carriers, complexation and liquid crystalline phases also maximize drug absorption [19].

Bioavailability Enhancers in Ayurvedic System of Medicine: In Ayurveda the concept of bio-enhancers is being used since centuries. Ayurveda had come with concept of reverse pharmacology in old time and now another Ayurveda-based technology of enhancing bioavailability of drugs is a remarkable milestone in field of medicines [20, 21]. Among the bio-enhancers, 'piperine' of black pepper was first demonstrated as the major part of "Yogvahi" [22]. There are following concepts [23-29] of bio-enhancers have been used in Ayurveda since time immemorial: 1. Yogvahi, 2. Anupaan, 3. Bhaishajya kala (proper time for taking medication in relation to food), 4. Bhawana, 5. Rasayan, 6. Sanshodhan (biopurification) and 7. Yog (formulation) & different kalpanas (various dosage forms)

Yogvahi: Concept of yogvahi for enhancing bioavailability is being used in Ayurveda since time immemorial [30, 31]. A very common example of yogvahi in Ayurveda include Pippali (Piper longum) and Maricha (Piper nigrum), which contains an important active compound named 'piperine' (1-piperoyl piperidine) which is responsible for bio-enhancing effect. Piperine is well established bio-enhancer used for potentiating the bioavailability and efficacy of many drugs including ingredients of vasaka leaves, vasicine, sparteine, sulfadiazine, rifampicin, phenytoin and propranolol [32-34].

Anupaan: Taking any type of medicament/drug before, with or after taking the principal (core) drug is known as Anupaan. It is another method to increase the bioavailability of drugs. Acharya Charaka mentioned that if, Anupaan is taken in proper manner then it will help in proper digestion & absorption of food material and therefore, ultimately increases the bioavailability of food nutrients. [35]

Bhaishajya Kala (Proper Time for Taking Medication): In Ayurveda there is description of proper Kala (time) for taking medication in relation to food. Acharya Sushruta mentioned 10 Kala (times) for taking medication in relation to food like Abhakta, Pragbhakta etc. Therefore, administration of drugs in such a way definitely modulate the bioavailbility of drugs.

Bhawana: It is special method for enhancing the bioavailability of drugs. In this method, the powder of one drug/drugs are triturated with the Swaras, Kwath etc. of another drug during the manufacturing of dosage forms to increase the effect of the drug. One important example of Bhawana Dravya includes 'Gomutra'. Gomutra is a well-established bio-enhancer of animal origin. Gomutra (cow urine) is mostly used in its distillated form than normal urine. It increases the bioavailability of antimicrobial, antifungal and anticancer agents[36]. Some other important Bhawana Dravya includes Ardraka Swaras, Nimbu (citrus) Swaras etc.

Rasayan: A group of very important drugs described in the Ayurvedic system of medicine named 'Rasayan'. Rasayan are known for their several properties, bioenhancing effect is one of them. The Rasayan drugs claimed for their

bioenhancing effects include, Madhuyashti (Glycrrhiza glabra), Draksha (Vitis vinifera), Lasun (Allium sativum), Ghritkumari (Aloe vera) etc.

Glycyrrhizin is a triterpenoid saponin found in Glycyrrhiza glabra (liquorice). Glycyrrhizin showed a more potent absorption enhancing activity than caproic acid at the same concentration tested. The absorption-enhancing activity obtained from the simultaneous treatment of sodium deoxycholate and dipotassium-glycyrrhizin was much greater than sodium deoxycholate alone in Caco-2 cell monolayers [37]. It also enhances cell division inhibitory activity of anticancerous drug `Taxol` by 5 folds against the growth and multiplication of breast cancer cell line. Inhibition of cancerous cell growth by Taxol in presence of glycyrrhizin was higher than treatment with taxol alone. It is reported that glycyrrhizin enchances the transport of antibiotics like rifampicin, tetracycline, nalidixic acid, ampicillin and vitamins B1 and B12 across the gut membrane [26].

Draksha (grape) contains a flavonoid glycoside in its fruit juice, named 'naringin' which makes grapefruit juice taste bitter. Naringin exerts a variety of pharmacological effects such as antioxidant, blood lipid lowering and anticarcinogenic activities. Also, naringin was reported to inhibit CYP3A1/2 and P-glycoprotein in rats [38,39].

Aloe vera is also proved as bio-enhancer. The results of two different Aloe vera preparations, i.e., whole leaf extract and inner filled gel indicate that the aloes improve the absorption of both the vitamin C and E. Aloe vera is very promising future nutritional herbal bio-enhancer [40].

Sanshodhan (Biopurification): Sanshodhan is a special type of modality described in Ayurvedic system of medicine. It is a type of biopurification used to eliminate the vitiated dosha (morbid humors) out of body as a preventive measure or to manage different disorders. Sanshodhan increases the strength of Agni, leading to increased digestion power and increased absorption of nutrients and drugs. Thus, ultimately increases the bioavailability of nutrients and drugs. In Ayurveda Rasayan (rejuvenative) and Vajikaran (aphrodiasic) drugs are advised to take after proper Sanshodhan (biopurification). Because proper biopurification increases the bioavailability of Rasayan and Vajikaran drugs, thus potentiates their efficacy.

Yoga/Formulations/ Kalpanas [Various Dosage Forms]: Plant based medicines of natural origin has been used by mankind since time immemorial. Ayurveda described different type of kalpanas (Swaras, Kwath, Phanta etc) and various formulations like Choorna, Avaleha, Ashava, Arishta, Guggulu, different minearal and herbo-mineral preparations. Initially in the Ayurveda, the physicians used the drugs in coarse crude form like Swaras, kwath etc. followed by fine crude form like Vati, Choorna and finer form like Bhasma etc. As the time passes the Ayurvedic physicians realize about the low bioavailability of drugs and this leads to the concept of various dosage form (from crude form to finer form). In finer form drugs got more absorption and ultimately the bioavailbility of drugs increases.

To alleviate many disorders bioavailability is best way to reduce dose, toxicity and cost of drug dosage form. Therefore, use of herbal bio-enhancers with the core drug is the best way to achieve this target. Uses of such agents are applicable not only for humans but also for animals in normal practices whether it is medicine or nutrition.

Mechanism of Action of Herbal Bio-enhancers: There are several mechanisms of action by which herbal bio-enhancers act. Different herbal bio-enhancers may have same or different mechanism of action. Among the various mechanisms of action postulated for herbal bio-enhancers some are as follows [10, 26]:

1. Reduction in hydrochloric acid secretion and increase in gastrointestinal blood supply.
2. By modulating the active transporters located in various locations eg. P-glycoprotein (P-gp). P-gp is an efflux pump which pumps out drugs and prevent it from reaching the target site. Bio-enhancers in such case act by inhibiting the P-gp.
3. Inhibition of gastrointestinal transit, gastric emptying time and intestinal motility.
4. Decreasing the elimination process thereby extending the efficacy of drug in the body by-Inhibiting the drug metabolizing enzymes like CYP 3A4, CYP1A1, CYP1B2 and CYP2E1 in the liver, gut, lungs and various other locations and by Inhibiting the renal clearance by preventing glomerular filtration, active tubular secretion by inhibiting P-gp and facilitating passive tubular reabsorption.
5. Modifications in GIT epithelial cell membrane permeability.
6. Bioenergetics and thermo-genic properties.
7. Suppression of first pass metabolism, inhibition of drug metabolizing enzymes and stimulation of gamma glutamyl transpeptidase (GGT) activity which enhances uptake of amino acids.

Benefits of Bio-enhancing: Bio-enhancers provide the following benefits [41]

- Reduced drug dosage: This can make the expensive drugs affordable by lowering the dose or dosing frequency ultimately reduce the toxic effects. Shortening the treatment period also increase the acceptance of patients mainly in case of chemotherapy.
- Increased efficacy.
- Increased Bioavailability.
- Therapeutic treatments which include heavy doses, accompanied by loss of metals and vitamins available in body, the bio-enhancers improve the nutritional status of body during the course of treatment.[2, 42]
- Reduced resistance of drug.
- Reduced adverse drug reaction or side effects.
- They improve oral absorption of wide range of nutrients such as vitamins, minerals, herbal extracts and amino acids.
- Decreases requirement of raw material for drug manufacture.

- Economically benefitted to the world economy by reducing the treatment cost.

Challenge and Hurdles: Since many decades bio-enhancers in drug delivery have been used successfully, but not all the approaches have been met with all success. Challenges have been encountered while the development of the concept of biopotentiation, to modify the physicochemical virtues of the drug likes drug degradation and crossing of biological barriers. The newly developed Bioenhancers have many challenges to be faced[43].

- To improve the properties of drug formulation like circulation in blood, increased functional surface area, protection of drug from degradation, crossing biological barriers & site specific targeting.
- The Research & development of herbal Bio-enhancers in large scale production is a problem. It is easy for pilot scale production than the large scale production.
- The next posed problem is on regulatory control. There is a need to have regulations for physicochemical & pharmacokinetic properties of newer bio-enhancer than the other conventional drug products.

Conclusion: In developing countries like India cost of treatment is the major concern for modern medicines. Scientific society has their eagle eyes on reduction of cost of dosage and indirectly the whole treatment. Bio-enhancers have decreased the usual dose so ultimately reduced the drug-resistance, toxicity and shortens the period of treatment. Therefore, reduced the cost of whole treatment. The traditional wisdom of Ayurveda can have immense utility in enhancing the bioavailability of allopathic (synthetic) drugs. If the herbal bio-enhancers co-administered with synthetic drugs then this co-administration provide very effective results & covered each and every class of drug. In this way, if the traditional wisdom of Ayurveda coupled with modern technologies, it would open new vistas in the public health care.

References

1. Patwardhan, B., Mashelkar, R.A. (2009). Traditional medicine inspired approaches to drug discovery and development: can Ayurveda show a way forward. *Drug Discov. Today*, 14, 804-811.
2. Kang, M. J., Cho, J.Y., Shim, B.H., Kim, D.K., Lee, J. (2009). Bioavailability enhancing activities of natural compounds from medicinal plants. *J. Med. Plants Res.*, 3(13), 1204-1211.
3. Middleton, E. Jr., Kandaswami, C., Theoharides, T.C. (2000). The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacol. Rev.*, 52, 673–751.
4. Navin, P., Atal, B., Bedi, K.L. (2010). Bioenhancers: Revolutionary concept to market. *J. Ayur. Intergrat. Med.*, 1, 96-99.
5. Havsteen, B.H. (2002). The biochemistry and medical significance of the flavonoids. *Pharmacol. Ther.*, 96, 167–202.
6. Zhang, S., Morris, M.E. (2003). Effects of the flavonoids biochanin A, morin, phloretin, and silymarin on P-glycoprotein-mediated transport. *J. Pharmacol. Exp. Ther.*, 304, 1258–1267.
7. Feng, H.P., Huang, C.L., Zhang, L.T., Li, R.F. (2004). Pharmacokinetics and bioavailability of cinnamic acid in mice. *J. China Pharm. Univ.*, 35, 328-330.

8. Ratndeep, Singha, Sarita, Devib, Jatin, H., Patela, Urvesh, D., Patela et al.(2009). Indian Herbal Bioenhancers: A Review, *Phcog Rev*, 3(5), 80-82.
9. Annamalai, R., Manavalan, R. (1990). Effect of Trikatu and its individual components and piperine on gastrointestinal tract. Trikatu—A bioavailability enhancer. *Indian Drugs*, 27, 595- 604.
10. Muttepawar, S.S., Jadhav, S.B., Kankudate, A.D., Sanghai, S.D., Usturge, D.R., (2014). A Review on Bioavailability Enhancers of Herbal Origin. World. *Journal of Pharmacy and Pharmaceutical Sciences*, 3(3), 667-677.
11. Kritika, Kesarwani, Rajiv, Gupta (2013). Bioavailability enhancers of herbal origin: An overview. *Asian Pacific Journal of Tropical Biomedicine*, 3(4), 253-266.
12. Bharat, Jhanwar, Somdatt, Gupta (2014). Biopotentiation using Herbs: Novel Technique for Poor Bioavailable Drugs. *IJPRIF*,6(2), 443-454.
13. Utsunomiya, T., Kobayashi, M., Pollard, R. B., Suzuki F. (1997). Glycyrrhizin, an active component of licorice roots, reduces morbidity and mortality of mice infected with lethal doses of influenza virus. *Antimicrobial Agents and Chemotherapy*, 41(3) 551–556.
14. Crance, J. M., Scaramozzino, N., Jouan, A., Garin D. (2003). Interferon, ribavirin, 6-azauridine and glycyrrhizin: antiviral compounds active against pathogenic flaviviruses. *Antiviral Research*, 58(1) 73–79.
15. Atal, N., Bedi K. L. (2010). Bioenhancers: revolutionary concept to market," *Journal of Ayurveda and Integrative Medicine*, 1(2), 96–99.
16. Lundin, S., Artursson, P. (1990). Absorption enhancers as an effective method in improving the intestinal absorption. *Int J Pharm*, 64, 181-186.
17. Aungst, B.J., Blake, J.A., Hussain, M.A. (1991). An in-vitro evaluation of metabolism and poor membrane permeation impeding intestinal absorption of leucine enkephalin and methods to increase absorption. *J Pharmacol Exp Ther*, 259, 139-145.
18. Varma, M.V., Ashokraj, Y., Dey, C.S., Panchagnula, R. (2003). P-glycoprotein inhibitors and their screening: a perspective from bioavailability enhancement. *Pharmacol Res*, 48, 347-359.
19. Sushma, Drabu, Smriti, Khatri, Sheveta, Babu, Pratush, Lohani (2011). Review Article - Use of Herbal Bioenhancers to Increase the Bioavailability of Drugs. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 2(4), 107-119.
20. Patwardhan, B., Mashekar, R.A. (2009).Traditional medicine inspired approach to drug discovery and development: can Ayurveda show a way forward?. *Drug discovery today*, 14, 804-811.
21. Junginger (1996). Drug Absorption Enhancement: Concepts, Possibilities, Limitations and Trends. *Journal of drug targeting*, 4(1), 51-52.
22. Johri, R.K., Zutshi, U. (1992). An Ayurvedic formulation 'Trikatu' and its constituents. *Journal of Ethnopharmacology*, 37, 85-91.
23. Sharma P.V. (2003). Dravya guna vigyan. 2nd ed. Varanasi: Chaukhambha bharati academy, (chapter 4).
24. Mishra Siddhinandan (2003). Abhinav bhaishajya kalpana vigyan. 1st ed. Varanasi: chaukhambha surbharati prakashan, (chapter 14).
25. Sushruta (2004). Sushruta Samhita. 1st ed. Varanasi: chaukhambha sanskrit sansthan, (Uttar Tantra- chapter 64).
26. Agnivesha, Charaka, Dridhabala (2002). Charaka Samhita. 1st ed. Varanasi: Chaukhambha bharati academy, (Kalpa Sthan- chapter 12).
27. Agnivesha, Charaka, Dridhabala (2002). Charaka Samhita. 1st ed. Varanasi: Chaukhambha bharati academy, (Chikitsa Sthan- chapter 1).
28. Agnivesha, Charaka, Dridhabala (2002). Charaka Samhita. 1st ed. Varanasi: Chaukhambha bharati academy, (Sutra Sthan- chapter 4).
29. Agnivesha, Charaka, Dridhabala (2002). Charaka Samhita. 1st ed. Varanasi: Chaukhambha bharati academy, (Sutra Sthan- chapter 16).
30. Singh, J., Dubey, R.K., Atal, C.K. (2002). Piperine-mediated inhibition of glucuronidation activity in isolated epithelial cells of the guinea-pig small intestine:

evidence that piperine lowers the endogeneous UDP-glucuronic acid content. *J Pharmacol Exp Ther.*, 302, 645–50.
31. Johri, R.K., Zutshi, U. (1992). An Ayurvedic formulation, Trikatu and its constituents. *J Ethnopharmacol.*, 37, 85–91.
32. Atal, C.K., Zutshi U, Rao, P.G. (1981). Scientific evidence on the role of Ayurvedic herbals on bioavailability of drugs. *J. Ethnopharmacol*., 4, 229-232.
33. Bano, G., Amla, V., Raina, R.K., Zutshi, U., Chopra, C.L. (1987). The effect of piperine on pharmacokinetics of phenytoin in healthy volunteers. Planta Med., 53,568-569.
34. Bano, G., Raina, R.K., Zutshi, U., Bedi, K.L., Johri, R.K., Sharma, S.C. (1991). Effect of piperine on bioavailability and pharmacokinetics of propranolol and theophylline in healthy volunteers. *Eur. J. Clin. Pharmacol*., 41,615-617.
35. Agnivesha, Charaka, Dridhabala (2002). Charaka Samhita. 1st ed. Varanasi: Chaukhambha bharati academy, (Sutra Sthan- chapter 27).
36. Kekuda, P. T., Nishanth, B. C., Praveen Kumar, S. V., Kamal, D. Sandeep, M., H. K., Megharaj (2010). Cow urine concentrate: a potent agent with antimicrobial and anthelmintic activity. *Journal of Pharmacy Research*, 3, 1025–1027.
37. Imai, T., Sakai, M., Ohtake, H., Azuma, H., Otagiri, M. (2005). Absorption enhancing effect of glycyrrhizin induced in the presence of capric acid. *Int J Pharm.*, 27:11–21.
38. Zhang, H., Wong, CW., Coville, P.F., Wanwimolruk, S. (2000). Effect of the grapefruit flavonoid naringin on pharmacokinetics of quinine in rats. *Drug Metabol Drug Interact*, 17, 351-363.
39. Lim, S.C., Choi, J.S. (2006). Effects of naringin on the pharmacokinetics of intravenous Paclitaxel in rats. *Biopharm Drug Dispos*, 27, 443-447.
40. Vinson, J.A., Kharrat, H.A., Andreoli, L. (2005). Effect of Aloe vera preparations on the human bioavailability of vitamin C and E. *Phytother Phytopharmacol*., 12, 760–765.
41. Prasad, K. V., Laxdal, A., Yu, M., Raney, B. L., (1995). Antioxidant activity of allicin, an active principle in garlic. *Molecular and Cellular Biochemistry*, 148(2), 183–189.
42. Khan, I. A., Mirza, Z. M., Kumar, A., Verma, V., Qazi, G. N. (2006). Piperine, a phytochemical potentiator of ciprofloxacin against Staphylococcus aureus. *Antimicrobial Agents and Chemotherapy*, 50(2), 810–812.
43. Fatima, X., Grace et. al. (2013). Bioenhancers–A New Approach in Modern Medicine. *Indo American Journal of Pharmaceutical Research*, 3(12), 1576-1580.