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Molecular targets of pepper as bioavailability enhancer

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SUMMARY

Black pepper (family Piperaceae), is called king of spices because it is one of the oldest spice and alone accounts for about 35% of the world's total spice trade. The pepper is used in Ayurvedic medicine for the treatment of various ailments particularly neurological, broncho-pulmonary and gastrointestinal disorders. Pepper has also been reported to have various pharmacological actions but recently, it is highlighted as a bioavailability enhancer. This results in higher plasma concentration of drugs, nutrients, ions and other xenobiotics, rendering them more bioavailable for physiological as well as pharmacological actions in the body. Numerous scientific studies reported that piperine; a main bioactive compound of pepper, is responsible for its bioavailability enhancing property. It's a well known fact that pepper enhances bioavailability by inhibition of microsomal enzyme system but other mechanisms are also responsible to acts as a bioavailability enhancer. The brief overview of the mechanism of action of pepper as well as its applications as bioavailability enhancer is given in the present article.

Key words: Piperine; Bioavailability enhancer

INTRODUCTION

Pepper species present as one of the key component in many preparations and formulations in traditional Ayurvedic medicine used for various ailments particularly neurological, broncho-pulmonary and gastrointestinal disorders (Raj *et al*., 1978; Johri *et al*., 1992). Black pepper is known as king of spices because it is one of the oldest spices and alone accounts for about 35% of the world's total spice trade (Majeed *et al*., 2000). Piperine (a pungent alkaloid) belongs to family Piperaceae, is the major constituent of these plants and its content is 5 - 9% and 3 - 5% (on dry weight basis) in *P. nigrum* Linn (black pepper) and *P. longum* Linn (long pepper) respectively. It is isolated from fruits and it is absent in the leaves and stem of plants. Other pungent alkaloids which occur in pepper species in small amount are chavicine, piperidine and piperrettin. The sharp flavor of freshly ground pepper is attributed to the compound chavicine, a geometric isomer of piperine. The loss of flavor of pepper on storage is associated with slow trans-formation of chavicine into piperine (Anonymous, 1989). Pepper has been reported to have various pharmacological actions like immunomodulatory, antitumor (Sunila *et al*., 2004), analgesic, anti-inflammatory (Annamalai *et al*., 1990), antiulcer (Raffaele *et al*., 2002), antidiarrheal (Bajad *et al*., 2001) etc. but recently, it is highlighted as a bioavailability enhancer and this property of pepper results in

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higher plasma concentration of drugs, nutrients, ions etc., rendering them more bioavailable for physiological as well as pharmacological actions in the body.

In Ayurveda, black pepper, long pepper and ginger often used together in equal proportions in a formulation known as 'trikatu'. Trikatu is a Sanskrit word meaning three acrids. According to Ayurveda, the three acrids collectively act as "kaphavatta-pitta-haratwam" which means "correctors of the three humors (doshas) of the human organism" (Johri *et al*., 1992). The use of Trikatu or its ingredients is traditionally well-known in the treatment of a variety of gastrointestinal disorders, and all three acts to improve digestion. Out of 370 compound formulations listed in the Handbook of Domestic Medicines and Common Ayurvedic Remedies, 210 contain either trikatu or its individual ingredients like long pepper or black pepper. Experimental evidence shows that the main purpose of the use of trikatu, and/or its constituents individually as well as collectively into numerous Ayurvedic formulations was most probably to enhance the efficacy of pharmacologically active ingredients (Johri *et al*., 1992). The first scientific study carried out at the Regional Research Laboratory, Jammu-Tawi in India by Atal *et al*. (1981) to confirm the bioavailability enhancing property of pepper. They found that there was increase in blood levels of vasicine by 232% and sparteine by more than 100% in *Pepper longum* treated group as compare to control animals. Based on studies carried out in animals as well as human volunteers, it was noted that the bioavailability enhancing property of pepper was attributed to its principle alkaloid, piperine.

MATERIALS AND METHODS

Mechanism of bioavailability enhancement by the pepper

Pepper has been shown to increase the bioavailability and efficacy of large number of synthetic as well as natural compounds. Extensive research was carried out to find out mechanisms of pepper in bioavailability enhancement. Our body has several mechanisms to control the exposure of its cells to nutrients & other substances. Four of these mechanisms are important with regard to pepper: *assisted absorption, metabolic conversion, assisted exclusion and solubilizer attachment* (Russell, 2003). Piperine has remarkable ability to manipulate all four of these mechanisms to act as bioavailability enhancer (Fig. 1).

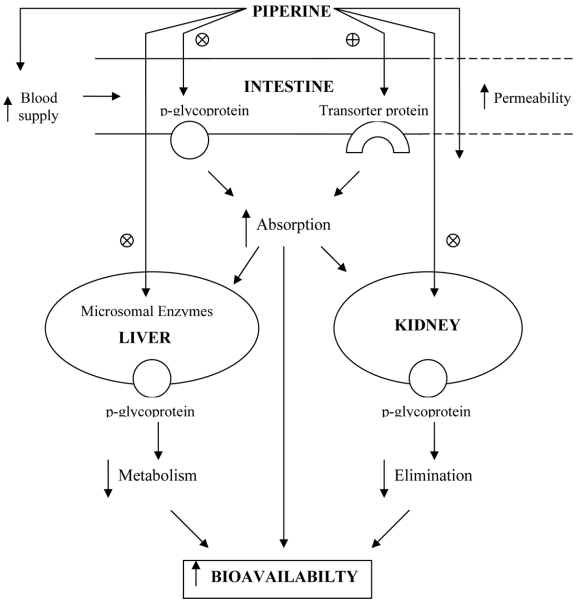

Fig. 1. Schematic diagram of molecular targets of Piperine as bioavailability enhancer.

Assisted absorption

This involves the use of transporter proteins in the digestive tract. These proteins actively transport the substances into the intestinal lining; from there they can be transferred further into the blood stream. Assisted absorption is particularly important to the amino acids in adequate amount in the body. Piperine stimulates amino acid transporters (Leucine amino peptidase and glycyl-glycine dipeptidase) activity due to alteration in enzyme kinetics in intestinal wall. A recent study using human intestinal

cells as an experimental model, showed that piperine by inducing alteration in membrane dynamics and permeation characteristics, along with induction in the synthesis of proteins associated with cytoskeletal function, resulting an increase in microvilli length (small intestine absorptive surface), thereby assisting efficient permeation through the epithelial barrier. The results also suggested that piperine is absorbed very fast across the intestinal barrier and it may modulate membrane dynamics due to its easy partitioning thus helping an efficient permeability across the intestinal wall (Khajuria *et al*., 1998; Khajuria *et al*., 2002). In conclusion, it was suggested that piperine by acting as an apolar molecule and forming apolar complex with drugs and solutes, alter the membrane lipid dynamics and change in confirmation of intestinal enzymes. Besides these specific mechanisms, some non-specific mechanisms are also reported to be involved in promoting rapid absorption of drugs and nutrients by the piperine, e.g. increased gastrointestinal blood supply, decreased hydrochloric acid secretion, increased emulsifying content of the gut, increased enzymes like gamma-glutamyl transpeptidase which participate in active and passive transport of nutrients to the intestinal cells (Annamalai *et al*., 1990; Johri *et al*., 1992).

Metabolic conversion

This mechanism involves the use of enzymes to convert substances (substrates) into different substances (metabolites) that may be biologically less active and/or, are more easily carried in the blood to kidneys for easy excretion. Piperine inhibits number of enzymes responsible for metabolism of drugs as well as nutrients. The studies suggested that piperine nonspecifically inhibited many Cytochrome P_{450} isoforms -CYP3A4 (main drug metabolizing microsomal enzyme) hepatic arylhydrocarbon hydroxylse, UDP-glucuronyltransferase and other enzymes involved in biotransformation of drugs and other xenobiotics (Atal *et al*., 1985; Singh *et al*., 1986; Bhardwaj *et al*., 2002). Moreover, piperine administration in rats resulted in 50% decrease of total Cytochrome P_{450} content indicating a suicide inhibition by piperine (Dalvi, 1991). Thus, by inhibiting the microsomal enzyme system, piperine protects the drug from being metabolized/ oxidized in its first pass passage through the liver after being absorbed. Moreover, the prolongation of bioavailability duration depends on the duration of inhibition of Cytochrome P3A4 in liver by piperine (Bhardwaj *et al*., 2002).

Assisted exclusion

This involves the use of transporter proteins that pump certain substances out of the cells, whereupon they can be taken away from the cells by the blood. On one hand, the activities of these pumps protect cells from toxic overloads of many poisonous substances, on other hand; they can also decrease the efficacy of many beneficial drugs and supplements by pumping out these substances from the cells before they can act. One of the most important such pump proteins is *p-glycoprotein (Pgp)*, which is found in the membrane of the cells in the intestine, brain, liver, pancreas, kidneys and other tissues. Piperine inhibits this *Pgp* and thereby preventing elimination of drugs, making them more bioavailable to the tissue (Johri *et al*., 1992).

Solubilizer attachment

This prevents substances from entering cells by linking them chemically to a highly water-soluble substance. This not only alters the biological activity of the substance but also make the hydrophilic complex unable to diffuse through the cell membrane. One of the important solubilizer found in the body is glucuronic acid. Substances bound to this solubilizer are usually excreted into the urine or into the small intestine, depending upon the nature of the substance. The study carried out using isolated guinea pig epithelial cells, demonstrated that piperine modified the rate of glucuronidation by lowering the endogenous UDP-glucuronic acid content and also by inhibiting UDP - glucuronyl-

transferase activity (Majeed *et al.*, 1998). In this way, pepper permitting more of the substances to enter the body in active form.

Thus, it appears that the pepper acts as bioavailability enhancer either by promoting rapid absorption from the gastrointestinal tract, or by protecting the drug from first pass metabolism in liver after being absorbed, or by a combination of these two mechanisms.

Due to its well known bioenhancing property, pepper has been tested as well as used in combination of many allopathic drugs. The long term oral administration of propranolol is rendered to be difficult because steady therapeutic level of this drug is not achieved or maintained. Moreover, high dose is needed for efficacy which will lead to adverse events. Pepper caused two fold increases in bioavailability of propranolol by inhibiting its metabolism by the liver and thereby excellent antihypertensive effect was obtained with low dose of propranolol (Bano *et al.*, 1991). Similar results were also obtained when piperine was administered along with theophylline, an anti-asthmatic drug and phenytoin, an anti-epileptic drug and these were due to inhibition of biotransforming enzymes in liver as well as slowing down the elimination rate (Bano *et al.*, 1978; Bano *et al.*, 1991). Piperine coadministered with a formulation containing rifampicin, pyrazinamide and isoniazid has been tested in human volunteers where, the comparative levels and peak concentration of these drugs were higher in the presence of piperine. This resulted in the development of effective anti-tuberculosis and anti-leprosy formulations which are cost prohibitive (Zutshi *et al.*, 1989). Likewise, co-administration of piperine significantly enhanced bioavailability of beta-lactam antibiotics, amoxicillin trihydrate and cefotaxime sodium in rats (Hiwale *et al.*, 2002). The piperine also increased the bioavailability and thereby potentiated the analgesic activity of non-steroidal anti-inflammatory agents like nimesulide. This will lead to reduction in the dose and dose-related side effects (Amarjit *et al.*, 2000). The drug sildenafil is metabolized mainly by the enzyme CYP3A4, an enzyme found in many tissues, on its way to the blood stream. Piperine, an inhibitor of CYP3A4 enzyme, boosts the efficiency of sildenafil by 2.5 times and also prolong its action, probably by about 2 h and thereby, increase efficacy of sildenafil in erectile dysfunction (Russel, 2003). Human Immuno Deficiency Virus (HIV) protease inhibitors have shown reduced effectiveness because of p-glycoprotein activity. By inhibiting p-glycoprotein, piperine increases the residence time of protease inhibitors in the cells and thereby increases their effectiveness in Acquired Immuno Deficiency Syndrome (AIDS) (Russell, 2003). Piperine, being an inhibitor of p-glycoprotein, prevents the efflux of ciprofloxacin from the cells of *S. aureus*, thereby, potentiating the antibacterial activity of the fluoroquinlone (Inshad *et al.*, 2006).

This successful use of pepper to increase bioavailability of different drugs has created an interest in the area of absorption of nutrient and food supplements because nutritional deficiencies due to poor gastrointestinal absorption is increasing problem not only developing countries but developed countries also. Recently, as a result of efforts in this direction, a formulation called Bioperine® (Sabinsa Corporation) has been formulated. Bioperine is a standardize extract of fruits of *P. nigrum* Linn and *P. longum* Linn and contains 95% of piperine. Co-administration of Bioperine® enhance bioavailability of nutrients like Beta-carotene, Coenzyme Q_{10}, Selenium, Vitamin B_6, Vitamin C, amino acids and herbal extract like Curcumin (Majeed *et al.*, 1999). Oral administration of Bioperine® (20 mg & 5 mg, respectively) along with 2 g Beta-carotene and 120 mg Coenzyme Q_{10} for 14 and 21 days, respectively showed two fold increased blood level of Beta-carotene (Fig. 2) (Badmaev *et al.*, 1999) and 30% greater Area Under Curve (AUC) of Coenzyme Q_{10} in human volunteers (Fig. 3) (Badmaev *et al.*, 2000). Similarly, Bioperine® (5 mg) showed increase in serum levels of selenium as well as serum Vitamin-

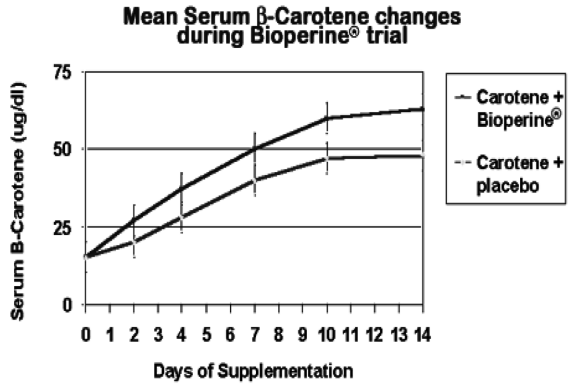

Fig. 2. Effect of Bioperine® on mean serum Beta-carotene level in human volunteers (Badmaev *et al.*, 1999).

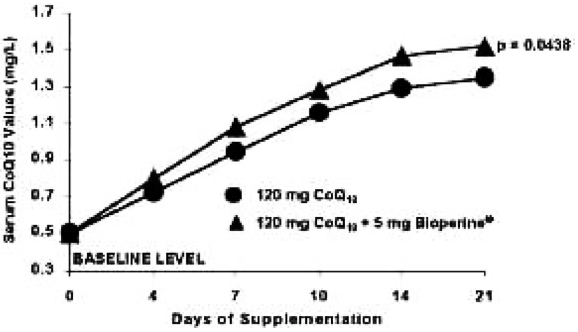

Fig. 3. Effect of Bioperine® on mean serum CoQ_{10} level in human volunteers (Badmaev *et al.*, 1999).

B_6 absorption at the end of 6 weeks and 4 h treatment period respectively as compare to placebo in human volunteers (Majeed *et al.*, 1999). The medicinal properties of curcumin cannot be utilized because of its poor bioavailability due to its rapid metabolism in liver and intestinal wall. Concomitant administration of 20 mg piperine enhanced bioavailability of curcumin by 2,000% through inhibition of hepatic and intestinal glucuronidation in both animals and humans without adverse effects (Fig. 4) (Shobha *et al.*, 1998). Piperine also enhanced the bioavailability of the tea polyphenol(-)-epigallocatechin-3-gallate in mice (Lambert *et al.*, 2004). The proposed mechanism behind the bioenhancing property of piperine, especially in nutrient and food absorption, is the induction of 'thermogenesis', a process of the heat

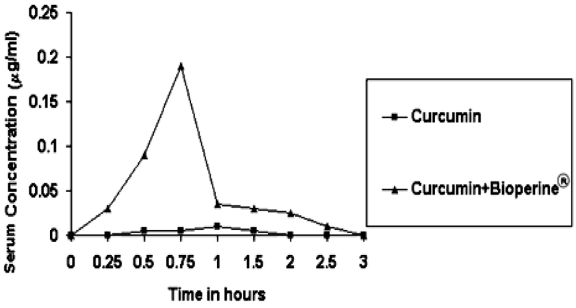

Fig. 4. Effect of Bioperine® on serum concentration of curcumin in Human volunteers (Shobha *et al.*, 1998).

production associated with digestion of food. Mostly, food-induced thermogenesis is facilitated by the beta receptors. Activation of beta receptors increases the level of cAMP, which activates enzymes resulting in biochemical reactions, which produce the heat energy. Piperine has been found to stimulate the release of epinephrine, which in tern activate this cascade of thermogenesis (Kawada *et al.*, 1998; Margriet *et al.*, 2006). Besides this mechanism, recent study suggested that piperine induced release of pancreatic enzymes, amylase and lipase as well as intestinal enzymes, trypsin and chymotrypsin, may also play a role to promote the digestion and efficacy of both nutrients and herbal drugs (Patel *et al.*, 2000). Since, very small oral dose of piperine (few milligrams) has the profound effects on nutrient absorption, piperine is termed as 'supernutrient' and based on its possible thermogenic effects on body, it might be considered as 'thermonutrient' (Walker, 1997).

Mostly, pepper is known for its bioenhancing effect but it also has ability to cause enzyme induction and thereby increase metabolic rate in the body. This will lead to decrease bioavailability of drug in some cases but fewer evidences are there which support this statement. The decline in serum concentrations and bioavailability of diclofenac sodium and isoniazid were observed when they were used along with Trikatu (Karan *et al.*, 1998; Lala *et al.*, 2004). Piperine induced decrease in gastrointestinal motility and delay in the gastric

emptying time lead to decrease in the absorption of isoniazid from the intestine (Karan *et al*., 1998). Moreover, in case of prodrug, metabolism leads to formation of more active compound. Pepper slows down the conversion of prodrug to its active metabolites and reduces its bioavailability. Thus, the activity of pepper is little bit complex and require thorough research. Based on this, pepper users should be cautious when pepper is used in combination of unusual substances as alteration in bioavailability might be resulted in undesirable side effects. This becomes very important as many physicians do prescribe modern as well as herbal drugs together for the treatment various diseases.

Piperine is effective and safe in a broader range. Studies showed that therapeutic dose of piperine to be between 3 mg to 399 mg with no adverse effects. The bioenhancing dose of piperine for the drugs is a maximum of approximately 15 mg/ person/day, or not more than 20 mg/day in divided doses. The dose of piperine required for increasing absorption of nutrients is several times lower than that commonly used to bioenhance blood level of drugs. Thus, the preferred oral dose range of piperine considered to be bioenhancing for absorption of nutrients and food supplements is calculated as 0.00004 - 0.15 mg/kg body weight. This dose is 4,000 times to 40,000 times less than the LD_{50} dose of piperine (Majeed *et al*., 1998). Moreover, it is pointed out that lower concentration of piperine is quite effective in offering significant inhibition of the monooxygenases than the higher ones (Rashmeet *et al*., 1991).

DISCUSSION

Today, bioavailability of drug seems to be a major concern of new drug development and its performance *in vivo*. Irrespective of few controversial data, experimental evidences clearly illustrate pepper as a bioavailability enhancer of drugs as well as nutrients. Pepper may used to overcome the problem of malnutrition by improving absorption of nutrition and food supplements. The bioavailability enhancing property of pepper helps to lower the dosage levels and shorten the treatment course which intern reduces the dose of highly toxic drugs, and thereby minimizing their adverse effects. Pepper also helps to achieve and maintained the steady therapeutic level and thereby make the treatment more effective. Besides that, it also offers cost effective treatment, which will be of great importance for developing countries. All these advantages will lead to better patient compliance. This article will encourage the readers to further studies on its unrevealed mechanisms as bioavailability enhancer. Further, it will also be useful to add Pepper in different formulations to increase their bioavailability and to reduce adverse effects of active ingredients.

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