

Expert Opinion

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Berberine: a potential phytochemical with multispectrum therapeutic activities

Parameswara Rao Vuddanda, Subhashis Chakraborty & Sanjay Singh[†]
Banaras Hindu University, Institute of Technology, Department of Pharmaceutics, Varanasi, India

Importance of the field: The use of traditional medicines of natural origin is being encouraged for the treatment of chronic disorders, as synthetic drugs in such cases may cause unpredictable adverse effects. Berberine, a traditional plant alkaloid, is used in Ayurvedic and Chinese medicine for its antimicrobial and antiprotozoal properties. Interestingly, current clinical research on berberine has revealed its various pharmacological properties and multi-spectrum therapeutic applications.

Areas covered in this review: An extensive search in three electronic databases (Unbound Medline, PubMed and ScienceDirect) and internet search engines (Scirus and Google Scholar) were used to identify the clinical studies on berberine, without any time constraints. This review elaborates the recent studies which reveal that with time, the drug has evolved with superior therapeutic activities. In addition, this review will also attract the attention of formulation scientists towards the issues and challenges associated in its drug delivery and the probable approaches that may be explored to help patients reap the maximum benefit of this potentially useful drug.

What the reader will gain: A relatively large number of studies discussed here have revealed the possible areas where this phytochemical constituent can exhibit its therapeutic activities in the treatment of chronic ailments or diseases including diabetes, cancer, depression, hypertension and hypercholesterolemia.

Take home message: The potential of the drug remains to be harvested by designing a suitable formulation that could overcome its inherent low bioavailability.

Keywords: berberine, lipids, multidrug resistance, P-gp, P-gp inhibitors, polymers

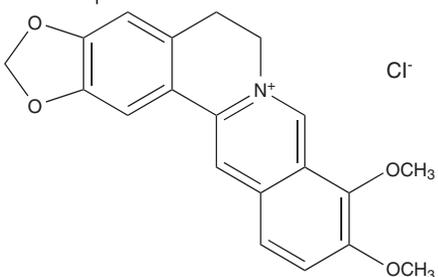
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1. Introduction

Recently, many research based pharmaceutical companies are investing relatively more time and money on development of herbal rather than synthetic drug formulations. This is because treatment of chronic diseases requires long-term drug therapy, and using synthetic drugs in such cases may cause unpredictable adverse effects. Because of this, even the WHO encourages use of traditional medicines of natural origin [1]. Plant extracts are the most widely used natural medicine due to their ease of availability and comparatively low production cost. The use of plant products as medicine in treatment of various chronic diseases or disorders is supported by Ayurveda, a system of traditional medicine native to the Indian

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Box 1. Drug summary.

Drug name	Berberine chloride
Phase	Phase III
Indication	Antibacterial, antiprotozoal, antidiabetic, anticancer, antidepressant; also used in treatment of cardiovascular disease
Pharmacology description/ mechanism of action	Inhibition of DNA repair mechanism, monoamine oxidase inhibitor, COX2 inhibitor
Route of administration	Oral and parenteral
Chemical structure	

Pivotal trials

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subcontinent and also practised in other parts of the world as a form of alternative medicine [2].

Berberine (Box 1) is a plant alkaloid with a long traditional history that is used both in Ayurvedic and Chinese medicine. This alkaloid is present in many plants, including *Hydrastis canadensis* (goldenseal), *Coptis chinensis* (Coptis or golden-thread), *Berberis aquifolium* (Oregon grape), *Berberis vulgaris* (barberry), *Tinospora cordifolia*, *Arcangelisia flava*, *Cortex rhellodendri*, *Rhizoma coptidis*, *Coptis japonica*, *Thalictrum minus*, *Berberis wilsonae* and *Berberis aristata* (tree turmeric). This phytochemical constituent can be found in the root, rhizome and stem bark of the plants. As a drug, it is traditionally used for its antimicrobial and antiprotozoal properties in Ayurvedic, Chinese and Middle-Eastern folk medicine. Specifically, Ayurveda describes berberine extracts and decoctions to have significant antimicrobial activity against a variety of organisms including bacteria, virus, fungi, protozoa, helminthes and *Chlamydia* [3]. Interestingly, current clinical research on berberine has revealed various other pharmacological properties and medicinal uses that are beneficial in treatment of chronic ailments or diseases including diabetes, cancer, depression, hypertension and hypercholesterolemia [4]. Thus, the drug has emerged as a medicinal agent with multispectral activities.

The pharmacology of berberine has been well reviewed in the past [4]. The aim of this section is, therefore, not to revisit in detail many of the reports that have been already covered, but rather to focus on recent studies which reveal that with time, the drug has evolved with superior therapeutic activities. In addition, the present review is also an attempt to attract the attention of the formulation scientists toward the problems

associated in its drug delivery and the promising approaches that could be explored to help patients reap the maximum benefit from this potentially useful drug.

2. Medicinal uses

2.1 Traditional uses

2.1.1 Antimicrobial activity

Berberine, usually available as chloride and sulfate salts, has a high bacteriostatic activity against *Staphylococcus epidermis*, *Neisseria meningitides* and *Escherichia coli*. One study has shown that berberine is specifically effective against cholera, giardia, shigella, and salmonella [5]. Another study, which supports the antimicrobial activity of berberine, has evaluated the microbial effect of the drug against 17 Gram-positive and -negative microorganisms on the basis of IC₅₀, MIC, minimum microbicidal concentration (MMC) and minimum microbistatic concentration (MMS). The IC₅₀ value obtained for *S. aureus* was 14.6 mg/ml, while for *Bacillus subtilis* it was 43 mg/ml. The high value in the latter case could be attributed to the development of resistance by the spores. The above study shows that berberine has marginal action on both Gram-positive and -negative organisms [6]. Materia Medica, a compilation of Chinese herbal medicines, indicates that berberine sulfate demonstrates significant antimicrobial activity against a wide range of microorganisms, including *Staphylococcus* (Staph), *Streptococcus* (Strep), *Candida* and *Salmonella*, as well as *Klebsiella*, *Clostridium*, *Pseudomonas proteus*, *Shigella*, *Vibrio*, *Cryptococcus* and *Entamoeba* spp. [7]. The National Institutes of Health has also reported that extracts of berberine have demonstrated significant antimicrobial activity against bacteria,

fungi, viruses and *Chlamydia*, confirming the antimicrobial activity of the drug [8].

2.1.2 Antiprotozoal activity

Berberine extracts and salts have demonstrated growth inhibition of *Giardia lamblia*, *Trichomonas vaginalis* [9] and *Leishmania donovani* [10]. The crude extracts of berberine have shown to be more effective than its salts [11]. In tropical climates, *Giardia lamblia* infestation (giardiasis) is a common occurrence, particularly in the pediatric population [12]. In a clinical trial, berberine administration improved gastrointestinal symptoms and resulted in a marked reduction in Giardia-positive stools; and it was effective at half the dose of the popular giardiasis medication, metronidazole [13]. A study has shown the drug's ability to markedly inhibit parasitic load and rapidly improve hematologic parameters in infected animals. *In vitro* results indicate that the drug has the ability to suppress organism maturation through inhibition of its multiplication, respiration and macromolecular biosynthesis of amastigote forms of the parasite, and interference with nuclear DNA of the promastigote form [10]. A randomized clinical trial in 215 patients has shown that pyrimethamine effect on chloroquine-resistant malaria was increased more by berberine (74%) than by tetracycline (67%) or cotrimoxazole (48%), which also indicates its antimalarial activity [14].

2.1.3 Antidiarrheal activity

Diarrhea caused by *Vibrio cholera* and *E. coli* has been the focus of numerous studies on berberine, and results indicate several mechanisms that may explain its ability to inhibit bacterial diarrhea. Berberine has been found to reduce the intestinal secretion of water and electrolytes induced by cholera toxin [15]. Other studies have shown that berberine directly inhibits some *V. cholera* and *E. coli* enterotoxins significantly, reduces smooth muscle contraction, intestinal motility and delays intestinal transit time in humans [16,17]. *In vitro* study indicates that berberine sulfate inhibits bacterial adherence to mucosal or epithelial surfaces, which is the first step in the infective process. This may be a result of berberine's inhibitory effect on fimbrial structure formation on the surface of the bacteria [18]. Another study in mice has shown that berberine has some activity against *E. histolytica*; this makes it useful against bilious disorders [19].

2.2 Potential therapeutic applications

2.2.1 Anticancer activity

In order to explore anticancer activity, the killing effect of berberine on nasopharyngeal carcinoma cells (NPC/HK1) was investigated. In this experiment, the cytotoxic effect of berberine in cell lines was assessed by using trypan blue exclusion assay. Surprisingly, berberine at 5 – 200 μM concentration was found to induce cell death in a dose-dependent manner. Treatment of cells with a 200- μM concentration of berberine for 5 h yielded a LD_{50} . The extent of DNA damage and repair after berberine treatment (0 – 100 μM) was also evaluated,

using comet assay. Administration of berberine up to 200 μM caused irreparable cell damage, as indicated by the increase in tail DNA content. However, the repair of DNA damage on this cell line in presence of H_2O_2 occurred within 1.5 h, indicating that berberine has contributed in the process of DNA repair inhibition which finally resulted in the cell death [20].

An *in vitro* cell viability study demonstrated that berberine improves As_2O_3 -mediated inhibition of glioma cell growth after 24-h incubation [21]. Here, the formation of a confluent layer of untreated control cells was observed that was inhibited upon incubation with a 5- μM concentration of As_2O_3 . The latter effect was even more pronounced in the presence of a 10- μM concentration of the drug. The As_2O_3 -mediated reduction in motility and invasion of glioma cells was enhanced upon co-treatment with berberine. Furthermore, it has been reported that PKC isoforms influence the morphology of the actin cytoskeleton, as well as activation of the metalloproteases MT1-MMP and MMP-2, which play a key role in cancer cell migration. Here, too, treatment of glioma cells with As_2O_3 and berberine significantly decreased the activation of PKC- α and - ϵ due to actin cytoskeleton rearrangements and blocking of the PKC-mediated signaling pathway. These results are interesting, as they indicate the potential use of berberine as a novel chemotherapeutic agent in the treatment of malignancy.

Another study has reported berberine's ability to inhibit *in vitro* cell proliferation in human prostate carcinoma cells. In this study, androgen-insensitive (DU145 and PC-3) and androgen-sensitive (LNCaP) prostate cancer cells were treated with berberine in a dose- (10 – 100 mmol/l) and time-dependent (24 – 72 h) manner. The drug exhibited inhibition of cell proliferation and induced cell death in both cases. The inhibition of proliferation may be due to its association with G1-phase arrest, inhibition of expression of cyclins D1, D2, E and cyclin-dependent kinase (Cdk) 2, 4 and 6 proteins. The drug also stimulated binding of Cdk inhibitors to Cdk, increased expression of Cdk -inhibitory proteins (Cip1/p21 and Kip1/p27), induced a higher ratio of Bax/Bcl-2 proteins, disrupted mitochondrial membrane potential, and activated caspase-9, caspase-3 and poly (ADP-ribose) polymerase, which together significantly enhanced apoptosis of DU145 and LNCaP cells. It was concluded that berberine induced apoptosis of human prostate cancer cells, mediated primarily through the G1 phase cell cycle arrest and caspase-dependent pathway. Treatment of non-neoplastic human prostate epithelial cells (PWR-1E) with berberine under identical conditions did not affect their viability [22].

The inhibitory effects of berberine on the proliferation and reproduction of certain tumorigenic microorganisms and viruses by transcriptional regulation, enzyme inhibition and interaction with both DNA and RNA has been well reviewed [23]. The drug's ability to suppress tumor growth and metastasis, and overcome multidrug resistance both *in vivo* and *in vitro*, clearly exhibits its potential in tumor chemotherapy.

2.2.2 Antidiabetic activity

Berberine has shown marked impact on carbohydrate and lipid metabolism. Recent preclinical and clinical studies suggest that it has a strong impact on glucose homeostasis. In fact, berberine increases insulin receptor mRNA expression through kinase C-dependent protein as promoter in cultured human liver cells and skeletal muscle [24].

Berberine has been shown to provide protection against β -cell damage and protection of pancreas from oxidative stress in diabetic rats. In an animal study, diabetic and hyperlipidemic condition was induced in rats by intraperitoneal injection of 35 mg/kg streptozotocin and administration of high-carbohydrate/high-fat diet. The overall experiment was conducted by using seven groups of rats consisting of diabetic untreated and treated rats with 75/150/300 mg/kg berberine, rosiglitazone 4 mg/kg and fenofibrate 100 mg/kg, and a control group. After 16 weeks of treatment, estimation of serum insulin level, insulin expression in pancreas, malonaldehyde content and superoxide dismutase activity in pancreas was carried out. It was observed that the diabetic rats showed alteration in pancreas to body weight ratio, insulin level, insulin sensitivity index, malonaldehyde content and superoxide dismutase activity. On the other hand, rats treated with 150 and 300 mg/kg berberine showed near control levels in the evaluating parameters. Moreover, in diabetic rat's mitochondrial vacuolization, swelling and dilatation of β -cell endoplasmic reticulum in pancreas was observed. The pancreatic islets were found to be atrophied and the number of secretory granules was decreased in diabetic rats, but less pathological change was observed in rats treated with 150 and 300 mg/kg berberine. These findings strongly suggest that berberine has a protective effect for diabetes through increasing insulin expression, β -cell regeneration, antioxidant enzyme activity and decreasing lipid peroxidation [25].

Yin and colleagues have demonstrated scientific evidence for the use of berberine in human beings to treat type 2 diabetes mellitus [26]. This clinical study was conducted on two age groups with type 2 diabetes mellitus. In study A, 36 adult subjects were selected with newly diagnosed type 2 diabetes mellitus and were randomly assigned to treatment with berberine or metformin (0.5 g three times daily) for 3 months. Periodically, the hemoglobin A1C, fasting blood glucose, postprandial blood glucose and plasma triglycerides were estimated. Significant decrease in hemoglobin A1c (from $9.5 \pm 0.5\%$ to $7.5 \pm 0.4\%$, $p < 0.01$), fasting blood glucose (from 10.6 ± 0.9 to 6.9 ± 0.5 mmol/l, $p < 0.01$), postprandial blood glucose (from 19.8 ± 1.7 to 11.1 ± 0.9 mmol/l, $p < 0.01$) and plasma triglycerides (from 1.13 ± 0.13 to 0.89 ± 0.03 mmol/l, $p < 0.05$) were observed in the berberine group. The drug showed similar hypoglycemic effect to that of metformin. In study B, 48 adult subjects with poorly controlled type 2 diabetes mellitus were supplemented with berberine for a period of 3 months and were clinically evaluated, similar to that in study A. Berberine was found to significantly decrease fasting blood glucose and postprandial blood glucose from week 1

until the end of the trial. Hemoglobin A1C decreased from $8.1 \pm 0.2\%$ to $7.3 \pm 0.3\%$ ($p < 0.001$), while fasting plasma insulin and homeostasis model assessments of insulin resistance index were reduced by 28.1 and 44.7% ($p < 0.001$), respectively. During the study, 20 (34.5%) patients experienced transient gastrointestinal-related adverse effects. Physiological functions of liver or kidney damage were not observed in any patient. Thus, this study indicates that berberine is a potent oral hypoglycemic agent that also has beneficial effects on lipid metabolism.

A recent study has investigated the molecular mechanism of berberine against insulin resistance, where the drug was found to increase insulin sensitivity through activation of insulin receptor (InsR) [24]. Berberine showed a dose- and time-dependent increase of InsR expression, InsR mRNA and protein expression in cultured human liver cells and L6 rat skeletal muscle cells. It was also observed that berberine enhanced InsR expression increases cellular glucose consumption only in the presence of insulin, and that berberine promotes InsR gene expression through a PKC-dependent activation of its promoter. Inhibition of PKC-abolished berberine caused InsR promoter activation and InsR mRNA transcription. In animal models, treatment of type 2 diabetes mellitus affected rats with berberine showed lowered fasting blood glucose and fasting serum insulin, increased insulin sensitivity and elevated InsR mRNA and PKC activity in the liver. In addition, berberine lowered blood glucose in KK-Ay type 2 but not in NOD/LtJ type 1 diabetes mellitus-affected mice that were insulin-deficient. The results suggest that berberine is a unique phytochemical constituent, active against insulin resistance in type 2 diabetes mellitus and metabolic syndrome.

2.2.3 Antidepressant activity

Recently, some neuropsychiatric research studies have investigated the CNS effects of berberine. Surprisingly, these studies demonstrated that berberine also possesses an antidepressant activity [27]. It was found that the drug affected the signaling pathway of L-arginine-NO cGMP, which manifested the antidepressant activity of the drug. The antidepressant activity was confirmed by conducting forced-swim test (FST) and tail-suspension test (TST) [28]. Total immobility period was recorded during a 6-min test. Berberine (5 – 20 mg/kg, i.p.) produced a reduction in immobility period in both tests. When berberine (5 mg/kg, i.p.) was co-administered with other typical antidepressant drugs such as mianserin (32 mg/kg, i.p.) or trazodone (2 mg/kg, i.p.), it was found to improve the anti-immobility effect of subeffective doses of the two antidepressants in FST but did not modify their effects. Berberine (5 mg/kg, i.p.) increased the levels of norepinephrine, serotonin or dopamine in the mouse whole brain, which was detected by using neurochemical analysis method.

In yet another study, the effect of berberine in FST and TST on mouse was investigated [29]. In this study, berberine

was administered in combination with atypical antidepressants with different mechanisms of action, including desipramine (noradrenaline [NA] reuptake inhibitor), serotonin (5-HT inhibitor), maprotiline (selective NA reuptake inhibitor), fluoxetine (selective 5-HT reuptake inhibitor) and moclobemide (monoamine oxidase [MAO] A inhibitor). The levels of these neurochemicals in mice striatum, hippocampus and frontal cortex were measured. The results show that berberine (10 and 20 mg/kg, p.o.) significantly reduced the immobility time during the FST and TST. Furthermore, berberine (20 mg/kg) increased NA and 5-HT levels in the hippocampus and frontal cortex. The research results support the view that berberine exerts antidepressant activity. The antidepressant mechanism of berberine may be related to the increase in NA and 5-HT levels in the hippocampus and frontal cortex.

2.2.4 Cardiovascular activity

2.2.4.1 Antihyperlipidemic activity

The metabolic effects of berberine have been widely investigated in recent years. In lipid metabolism, it has been observed that berberine was capable of lowering lipid concentration by increasing the transcriptional activity of LDLR promoter by a JNK pathway and stabilization of hepatic LDL-C receptor (LDLR) by an extracellular signal-regulated kinase (ERK)-dependent pathway [30,31]. Moreover, the influence on 5' AMPK and blocking of the MAPK/ERK pathway causes inhibition of lipid synthesis [32].

The antihyperlipidemic action of berberine has also been confirmed in humans [33]. In a clinical trial, 91 hypercholesterolemic people (52 males and 39 females) with types IIa and IIb hyperlipidemia were enrolled. Patients were divided into two groups: one group received 0.5 g berberine orally twice daily for a period of 3 months, while the other was maintained as a control group. At the end of treatment, blood samples were collected and fasting serum concentration of cholesterol, triglycerides, high- and low-density lipoprotein cholesterol (HDL-C and LDL-C), as well as liver and kidney functions, were examined. Cholesterol levels were lowered by 18%, triglycerides by 28% and LDL-C by 20%, without any significant change in HDL-C levels being observed, in 63 hypercholesterolemic patients. Moreover, berberine had no impact on kidney functions but improved liver function by reducing levels of alanine amino transferase, aspartate amino transaminase and gamma glutamyl transpeptidase enzymes. The control group showed no changes in any of the parameters examined. The results of the above clinical trial were re-evaluated in subjects who were neither on other drugs or herbs or nor on special diets before or during berberine therapy. In this study, 500 mg of berberine was administered twice daily to 32 hyperlipidemic patients for a period of 3 months. The antihyperlipidemic effect of the drug in this group was compared with 11 patients on placebo treatment. Berberine was found to significantly reduce the total cholesterol by 29%, triglycerides by 35% and LDL-C by 25%.

In another clinical study, berberine and a combination of berberine with policosanol, red yeast extract, folic acid and astaxanthin was orally administered daily to 40 subjects with moderate dyslipidemias divided in two parallel groups, each of 20 subjects. After a period of 4 weeks the total cholesterol, LDL-C, HDL-C, non-HDL, ApoB, ApoA, Lp(a) and triglycerides were estimated. Both berberine and the combination were found to significantly reduce TC (by 16 and 20%, respectively), LDL (by 20 and 25%), ApoB (by 15 and 29%) and TG (by 22 and 26%), as well as increasing HDL (by 6.6 and 5.1%). It may be concluded that food supplements containing natural products such as those studied could be a useful support to diet and lifestyle changes to rectify dyslipidemias and to reduce cardiovascular risk in subjects with moderate mixed dyslipidemias. Moreover, adverse events or impairments of liver transaminases were not observed in the study, indicating the tolerability and safety of the drug in humans [34].

2.2.4.2 Antihypertensive activity

The vasorelaxant effect of berberine has been observed in different animal models [35,36]. Berberine acts on both endothelium and underlying vascular smooth muscle to induce vasorelaxation via multiple cellular mechanisms. Although the mechanism of action of the drug on the vascular system is not clear, it has been proposed that at lower concentrations berberine-mediated aortic relaxation appears to be dependent on its effect on endothelium, while at higher concentrations the effects induced by the drug are independent of the presence of intact endothelium [37]. Other mechanisms involved have also been suggested that include ACE-inhibitor effect, direct release of NO/cGMP from rat aortic rings, increased sensitivity to the acetylcholine action, and activation of K⁺ channels [38-40].

A study has described the α_1 -adrenoreceptor-blocking activity of berberine [41]. In this study, berberine and prazosin (a known α_1 -adrenoreceptor blocker) were used wherein a parallel right shift in the phenylephrine cumulative dose-response curves were observed by both the drugs without any change in maximal response. In isolated rat anococcygeus muscle and rabbit aortic strip, the pA₂ was measured; pA₂ is an empirical measure of the activity (in concentration terms) of an antagonist that is not dependent on how the antagonist acts. The pA₂ is determined by measuring the value of the concentration ratio (r) at several antagonist concentrations, allowing an estimate of the antagonist concentration at which (r) would be 2. The pA₂ values of berberine were 6.62 and 6.54, respectively, while for prazosin it was 8.46 and 8.31, respectively. These results indicate that berberine has a competitive α_1 -adrenoreceptor-blocking action similar to that of prazosin.

A clinical study was carried out by 24- to 48-h ambulatory monitoring of 100 chronic heart failure and ventricular tachyarrhythmia patients. Berberine was found to decrease the frequency and complexity of ventricular premature

complexes and increase the left ventricular ejection fraction in chronic heart failure patients. In patients with ventricular tachyarrhythmia, berberine produced $\geq 50\%$ reduction in ventricular premature contraction in 62% and $\geq 90\%$ reduction in 38% of patients [42].

2.2.5 Anti-inflammatory activity

Inflammation is caused by prostaglandins (PGs) wherein COX-2 plays a key role in its synthesis. In an *in vivo* and *in vitro* study carried out in Wistar rats, berberine showed positive anti-inflammatory effects [43]. In this study, a 12-h berberine treatment at concentrations of 1, 10, and 100 mM in oral cancer cell line OC2 and KB cells showed reduced prostaglandin E2 (PGE2) production dose-dependently with or without 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (10 nM) induction. This berberine induced effect occurred rapidly after 3 h as a result of reduced COX-2 protein, but not enzyme activity. These *in vitro* anti-inflammatory effects were in agreement with the *in vivo* results where berberine pretreatment of Wistar rats inhibited the production of exudates and PGE-2 in carrageenan-induced air pouch. Finally, the authors concluded that berberine exhibits its anti-inflammatory effect through reduced COX-2 protein, but not through inhibition of enzyme activity.

3. Current status of therapeutic uses of berberine

Berberine is a traditional medicine that is prescribed in the Ayurvedic system, which has described its use as an antimicrobial, antidiarrheal and for treatment of various infections to be administered as *kvatha* (an Ayurvedic term for decoction) containing 5 – 10 ml of drug [44]. Presently, berberine is being used as a dietary supplement in the form of 100 – 200 mg capsule dosage per day in the United States; but it is not officially approved by the US FDA [45]. This drug is also abundantly used in Chinese medicine in dosage forms such as tablets and capsule form, at doses of 0.2 – 1.0 g/day for the treatment of various diseases, especially for type 2 diabetes mellitus [26].

The major clinical studies conducted so far to evaluate the pharmacological behavior of berberine are presented in Table 1.

4. Problems associated in drug delivery at intracellular level

Drug delivery through the oral route is the most preferable route of administration, especially to treat chronic diseases. Many drugs fail to meet their required therapeutic actions owing to poor bioavailability – which is usually due to low solubility, low permeability and/or high metabolism. Oral bioavailability of drugs majorly depends on their rate and extent of dissolution from the dosage form, their solubility in gastrointestinal fluid, *in vivo* stability and permeability of the

drug. The absorption process of drugs administered orally occurs mainly in the small intestinal region. Several mechanisms involved in drug absorption from the intestinal region to systemic circulation have been proposed, such as passive transcellular diffusion, paracellular transport, carrier-mediated transport and endocytosis. Usually, the passive diffusion mechanism is responsible for absorption of lipophilic drugs from the intestinal region, while the carrier-mediated transport mechanism is responsible for absorption of hydrophilic drugs.

Studies at the molecular level have revealed that several transporters are involved in the drug absorption process [46,47]. To date, the physiological, pathological and pharmaceutical functions of approximately 350 transporters have been extensively studied. These transporters include peptide transporters, amino acid transporters, organic cation transporters, organic anion transporters, bicarbonate transporters, glucose transporters, neurotransmitter transporters, ion transporters and exchangers, bile salt transporters, carboxylate transporters, urea transporters, amine transporters, folate transporters, fatty acid transporters, nucleoside transporters, phosphate transporters, nucleoside-sugar transporters and ABC transporters. It has also been recognized that these transporters are also involved in the active efflux of drugs that involve extrusion of the substrates outside the cell, which critically affect the drug absorption, disposition and elimination processes in the body. As many clinically used drugs – such as ACE inhibitors, anticancer and antiviral agents – are substrates of such transporters, the efflux system operated by the transporters should therefore be considered in order to optimize the oral bioavailability and decrease variability at the site of absorption [48,49].

Permeation glycoprotein (P-gp), an ATP binding cassette (ABC) transporter, has been identified as being responsible for active efflux of drugs used in the treatment of various chronic diseases [50]. P-gp, a 170-kDa plasma membrane glycoprotein, is abundant in columnar epithelial cells (enterocytes) in the lower gastrointestinal tract, canalicular surface of hepatocytes, apical surface of proximal tubules, capillary endothelial cells of brain, testis and in pregnant uterus [51,52]. The role of P-gp in the absorption process of drugs can be evaluated by the following processes: i) affinity of drugs towards P-gp; ii) the passive permeability of the drug molecules across the enterocytes; iii) expression levels of P-gp and in variability in expression levels along the gut; and iv) physiological variables that influence the solubility and passive transport along the gut [53]. However, the exact mechanism of efflux system operation is not clear. Usually, P-gp does not play an important role in the vital body functions; but on the basis of interaction with foreign bodies at membrane level (P-gp-mediated efflux system), it may be considered that P-gp has a defensive role in the body, such as in the active transport of cytotoxic compounds and endotoxins from intercellular to extracellular level [54,55]. On the other hand, overexpression of P-gp contributes to poor absorption of some therapeutically valuable drugs [56].

Table 1. List of major clinical trials conducted using berberine.

Sl No.	Activity studied	No. of subject/groups	Period of study	Parameters evaluated	Study outcome	Ref.
1	Antihyperlipidemic IIa and IIb category	91 hypercholesterolemic people (52 males and 39 females); placebo group, n = 28	3 months	C, TG, HDL-C and LDL-C	Lowered SC by 18%, TG by 28% and LDL-C by 20% but no difference in HDL-C level	[33]
2	Antihyperlipidemic	32 hyperlipidemic Asian patients; placebo group, n = 11	3 months	C, TG and HDL-C	Reduced TC level by 29%, TG by 35% and LDL-C by 25%	[33]
3	Antihyperlipidemic	40 Caucasian hyperlipidemic patients; placebo group, n = 20	4 weeks	TG and LDL-C	Reduced serum TG by 26% and LDL-C by 25% in combination dose and reduced serum TG by 22% and LDL-C by 20% in berberine dose	[34]
4	Antidiabetic	36 adults with newly diagnosed type 2 diabetes	3 months	Hemoglobin- α 1, FPG, postprandial glucose, FPI and HOMA-IR index	Significant reduction in hemoglobin- α 1 by 2%, FPG by 44%, PPG by 45%, fasting plasma insulin by 28% and HOMA-IR index by 44.7%	[26]
5	Antihypertensive	100 chronic heart failure patients (ventricular tachyarrhythmia)	24 – 48 h ambulatory monitoring	Ventricular premature contractions	> 50% reduction in ventricular premature contractions in 62% of patients and > 90% reduction in 38% of patients	[42]

C: Cholesterol; FPG: Fasting plasma glucose; FPI: Fasting plasma insulin; HDL-C: High-density lipoprotein cholesterol; HOMA-IR: Homeostatic model assessment – insulin resistance; LDL-C: Low-density lipoprotein cholesterol; PPG: Postprandial glucose; SC: Serum cholesterol; TG: Triglycerides.

At the same time, some studies have shown that the above fact has not been observed in all cases of drug absorption process, probably due to lack of affinity of the drugs with the P-gp transporters [57,58]. P-gp-mediated efflux creates the problem of drug absorption from the absorption site and thus causes alteration of a drug's pharmacokinetic behavior. Finally, it leads to failure of treatment or therapy due to build-up of multidrug resistance (MDR) for a particular disease. Based on the above discussion, it may be concluded that inhibiting the function of P-gp at various overexpression sites in the body can help to increase the rate and extent of absorption and permeation, and thus improve the bioavailability of drugs that are potential substrates of the P-gp-mediated efflux system [59-61].

5. Approaches to improve the bioavailability of berberine

Berberine chloride has poor bioavailability (< 5%), as its uptake is inhibited by the P-gp-mediated efflux system [62]; there is therefore a need to overcome the bioavailability issue of the drug to improve its therapeutic efficacy. This could be done by one of the following methods [63,64]:

- Development of novel non-P-gp substrates
- Administration of P-gp inhibitors with drug
- Designing formulations that allow the drug to bypass efflux pump transport.

The first method is quite tedious, and may consume great deal of time and money in attempting the synthesis of a new chemical entity. The second and third methods are relatively feasible due to advances in polymer science technology, which has resulted in the development of novel polymers for pharmaceutical applications. Fortunately, certain polymers have been studied that have the ability to inhibit the P-gp-mediated drug efflux system [65-68]. Interestingly, some lipid excipients, either in solid or liquid state, exhibit a similar property. However, the exact mechanism of P-gp inhibition by these unique excipients remains unclear. The different polymeric excipients reported to have the ability to inhibit the P-gp efflux system are polyethylene glycols (PEG), polypropylene oxanes (PPO), xanthan gum and sodium alginate [69]. The lipid excipients include peceol, gelucire 44/14 and cremophor EL [70]. Therefore, co-administration of drugs with suitable pharmaceutical excipients (P-gp inhibitors) in optimized concentration can help to improve the bioavailability and the possibility of drug delivery at the target site.

The determination of berberine in rat blood, liver and bile fluid carried out in a pharmacokinetic study suggests that berberine is metabolized in the liver and also undergoes hepatobiliary excretion [71]. Therefore, certain excipients such as lipids or formulations such as polymeric nanoparticulate drug delivery system – capable of bypassing the hepatic

metabolism, if administered in association with P-gp inhibitors – would help to increase the bioavailability of the drug [72,73]. Recently, cubic nanoparticles of ciclosporin A (also a P-gp substrate) were fabricated by using glyceryl monooleate/poloxamer 407. *In vitro* and *in vivo* studies (in Beagle dogs) were carried out using Neoral (ciclosporin A) as a reference product. Interestingly, the pharmacokinetic evaluation exhibited higher C_{max} (1371.18 ± 37.34 vs 969.68 ± 176.3 ng/ml), higher AUC_{0-t} (7757.21 ± 1093.64 vs 4739.52 ± 806.30 ngh/ml), and $AUC_{0-infinity}$ (9004.77 ± 1090.38 vs 5462.31 ± 930.76 ngh/ml). The relative oral bioavailability was 178% compared with the reference product, clearly indicating the role of P-gp inhibitors and cubic nanoparticle formulations in improving drug utilization, thereby improving its therapeutic efficacy [74].

6. Conclusion

The major objective of pharmaceutical research is either to increase the bioavailability or reduce the toxicity of a drug. The present review gives detailed information about the potential uses of berberine in relation to its multiple therapeutic actions, issues associated with drug delivery, and possible methods to improve the bioavailability of the drug. It is expected that this discussion will help the research community in this field to develop a platform for the design of an ideal delivery system for drugs affected by the P-gp-mediated drug efflux mechanism. At the same time, it would be useful to consider the extensive distribution of P-gp to determine which administration of formulations containing P-gp inhibitors may cause partial or reversible disturbance in the physiological system of the body. It is therefore important to carry out a detailed toxicological evaluation for such drug delivery systems.

7. Expert opinion

The above facts and figures indicate the multi-therapeutic activity of berberine, which may be explored in the routine treatment of various chronic diseases – especially in diabetes, cancer, hypertension and CNS disorders. However, low bioavailability of the drug is a matter of concern, as a very high dose is required to compensate the loss of drug via the P-gp efflux in the ileum region and achieve the required therapeutic plasma concentration. This indirectly exposes the body to an excess amount of drug and its related adverse effects.

Some researchers have administered berberine with ciclosporin A, which increased the absorption of berberine from the absorption site due to the P-gp-inhibiting ability of ciclosporin A [75]. As ciclosporin A by itself is an antibiotic with several side effects, this approach is not recommended to enhance the bioavailability of the drug. The only formulation so far developed is a microemulsion, prepared by using PEG and Tween-80 as polymer and surfactant, respectively. The

absorption behavior of the formulation in rat intestine was investigated. It was observed that the absorption of berberine from the microemulsion at the ileum region of intestine was significantly higher than that of raw medicine ($p < 0.01$) [76]. However, the basic issue of P-gp efflux associated with berberine still remains unanswered and is a challenge for pharmaceutical researchers.

A solution to this problem may be the development of a dosage form wherein the drug is encapsulated or conjugated with certain inactive and biodegradable pharmaceutical excipients capable of P-gp inhibition. These encapsulated or conjugated intermediates may then be administered as nanocolloidal systems including liposomes, niosomes, microspheres, nanoparticles and nanostructured lipid carriers. A nanocolloidal drug delivery system has the unique characteristic of

submicron-sized drug-encapsulated particles that can evade recognition by P-gp at the intestinal ileum region or tissue membrane. This dual approach would lead to enhanced systemic uptake or delivery at the targeted site with minimized drug loss as compared to the conventional dosage form [77].

Such a formulation could be used as a platform technology for the development of an effective dosage form for drugs that are P-gp substrates, thereby helping to achieve basic goals such as maximum therapeutic efficacy, minimum toxicity and reduced dose.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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Affiliation

Parameswara Rao Vuddanda,
Subhashis Chakraborty & Sanjay Singh[†]
[†]Author for correspondence
Banaras Hindu University,
Institute of Technology,
Department of Pharmaceutics,
Varanasi 221005, India
Tel: +91 542 670 2712; Fax: +91 542 236 8428;
E-mail: drsanjaysingh@rediffmail.com