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Research Article

Antimicrobial Profile of Antidiabetic Drug: Berberine

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ABSTRACT
Berberine is a plant alkaloid with long standing history of medicinal use in traditional Chinese, native American medicine as well as in indigenous Indian medicines. It is bright yellow coloured Iso quinolone alkaloid and is a chief alkaloid found in roots, stem and bark of bereberis species. It is procured from roots of B. aristata, B. petiolaris, B vulgaris, B.aquifolium, B. thumbergii, B. asiatica and hydrastis Canadensis.Among Chinese herbs it's primary sources are B sargentiana, Phellodendron amurense and Coptis chinensis from rhizomes and bark respectively.Berberine was demonstrated to have wide spectrum of pharmacological activities like anti hypertensive, anti inflammatory, anti oxidant, anti depressant, anti cancer, anti diarrhoeal, cholagogue, hepatoprotective and has also been used to treat oriental sores, trachoma, CHF. Most important of all its action is antimicrobial activity.

Keywords: Berberine, antimicrobial, antidiabetic

INTRODUCTION

Berberine is a plant alkaloid with long standing history of medicinal use in traditional Chinese, native American medicine as well as in indigenous Indian medicines. It is bright yellow coloured Iso quinolone alkaloid and is a chief alkaloid found in roots, stem and bark of bereberis species. It is procured from roots of B. aristata, B. petiolaris, B vulgaris, B.aquifolium, B. thumbergii, B. asiatica and hydrastis Canadensis.[1]

Among Chinese herbs it's primary sources are B sargentiana, Phellodendron amurense and Coptis chinensis from rhizomes and bark respectively.[2] Berberine was demonstrated to have wide spectrum of pharmacological activities like anti hypertensive[3], anti inflammatory[4], anti oxidant[5], anti depressant[6], anti cancer[7], anti diarrhoeal[8], cholagogue[9], hepatoprotective[10] and has also been used to treat oriental sores[11], trachoma[12], CHF[13]. Most important of all it's action is antimicrobial activity.

Recent studies have shown its effectiveness as anti diabetic in type 2 DM and hypolipidemic. The possible mechanisms for the antidiabetic action of berberine are[14,15]

1. Inhibition of alpha glucosidase activity
2. Enhanced insulin sensitivity
3. Increased glucose uptake by enhancing GLUT 4 translocation
4. Like biguanides it activates AMP activated protein kinase

Alkaloid berebrine has been added in the armamentarium of drugs used in DM type 2. It is no more a secret that India will be bearing the tag of DM capital of the world. DM is a disorder affecting multiple organs with propensity for infections of various kinds due to underlying mechanisms like hyperglycemia, oxidative stress etc. Hence it is going to be an added advantage to choose the drug for DM which has also got antimicrobial activity like berberine hydrochloride. None of the current anti diabetics can boast about additional antimicrobial action like berebrine hydrochloride.

Antibiotic resistance is a world health problem which is increasing at an alarming rate. Many frequently clinically used antibiotics like beta lactams ,aminoglycosides etc. are showing marked failure against antibiotic resistant strains like Methicillin resistant staph aureus (MRSA) and also vancomycin resistant enterocccus faecium (VREF)

This has increased nosocomial infection related health problems both in patients and medical professionals. Hence there is an urgent need for development of new antimicrobial drugs which have innovative and distinguished mechansms of action which would protect them against bacterial resistance.

Current available literature reveals that berberine hydrochloride has anti bacterial, antifungal, anti protozoal, antihelminthic and anti viral action.

1. Antibacterial spectrum covers Shigella, salmonella, klebsiella, Staph. aureus, Streptococcus pyrogenes, H pylori, Mycobacterium Tb, MDR Tb, K.pneumonias, E faecium, Trachoma (chlymadia trachomatis)
2. Antifungal - Candida species
3. Antiprotozoal - E. histolyticia, G.. lambia, T vaginitis, Leishmaniasis, Oriental sore

Antibacterial actions

Berberine hydrochloride was shown to reduce loss of water, sodium and chloride from rat ileum stimulated by

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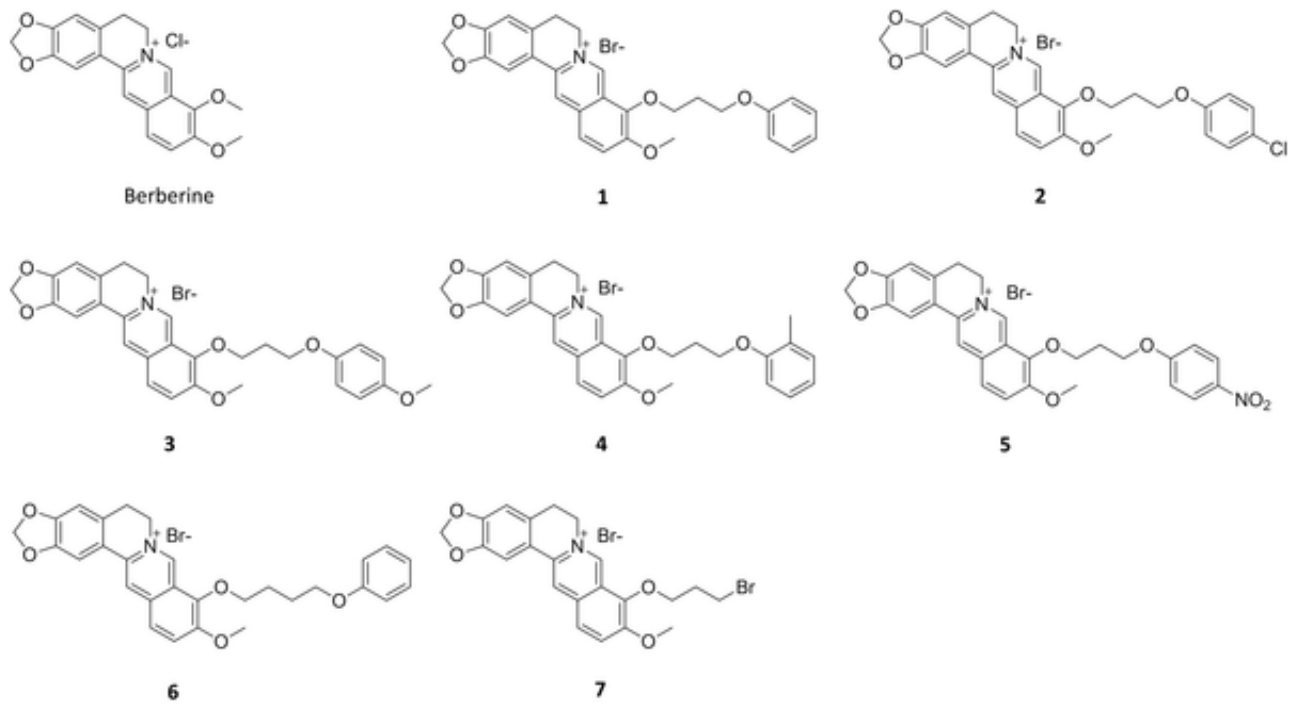

Figure 1. Chemical structures of berberine and its 9-phenoxyalkyl substituted derivatives.

cholera toxin .It was also shown to inhibit intestinal secretory response of enterotoxin of E. coli and V.Cholerae. Surprisingly it did not modify histological features of intestinal mucosa.[16]

Diarrheogenic intestinal infective organisms like Shigella dysentry, Salmonella paratyphi and various klebsiella species were attenuated by berberine. Adherence of Streptococcal pyrogen and E coli to host cells were shown to be blocked by Berebrine sulphate. Possibly this could explain mechanism of action of Berebrine against numerous pathogens.[17]

As berberine was proved to be active against gram positive bacteria but less active against gram negative one which initiated the study of the anti microbial activity of berberine derivatives. Berebrine was also shown to inhibit Helicobacterium pylori. It had also shown response against the multidrug resistant strains of TB bacilli[18]

The three protoberberine alkaloids were compared regarding their antimicrobial actions. Anti microbial effect of E. coli was more by berberine than copticine and least with palmitine.[19]

Possible mechanism by which berberine act as antimicrobial agent include supression of cell adhesion as mentioned earlier and migration. It is also proved to inhibit microbial enzymes. Berberine is a cationic molecule which binds to DNA and this DNA binding apparently contributes to antimicrobial activity of berberine. Berberine plant produces both antibacterial compounds and compounds which target bacterial efflux mechanisms. Anti microbial activity of berberine gets potentiated by 5 - Methoxy hydno carpine which suggests targeting of the bacterial efflux mechanisms.[20]

Microbial organisms protect themselves from synthetic and natural antimicrobials by defence mechanism known as multi drug resistant pumps (MDR). For these pumps amphipathic cations are preferred substrates. Berberine alkaloids which are produced by variety of plants are also readily extruded by MDR pumps they being cationic antimicrobials. NorA is drug efflux transporter or member of the facilitator family of drug antiporters that are widely spread among Gram negative, Gram positive bacteria and yeast. Several berberis medicine plants which produce berberine are also known to synthesize inhibitor of Nor A pump of staph aureus MDR pump. These inhibitors are identified as 5 Methoxy hydno carpine (5' mhc) and are amphipathic weak acids and they differ from cationic substrates of Nor A. Per se 5 mhc has no antibiotic activity but it is shown to strongly potentiate action of berberine and other Nor A substrates against staph aureus. 5' mhc inhibited MDR dependent efflux of berberine from S aureus cells which has resulted in to increased accumulation of berberine in the cells. This whole process helps to disable bacterial protective mechanism against berberine sensitive antimicrobial.[21]

Role of Filamentary temperature mutants(Ft Sz) in bacterial cell division –

Recent literature reports demonstrated that berberine is active against gram positive bacteria with minimum inhibitory concentration values (MIC) in the range of 100-400 µg /ml by targeting cell division protein Filamentary temperature mutants Z (FtsZ). [20] Filamentary temperature sensitive mutants Z (FtsZ) which are analogous to eukaryotic tubulin are essential bacterial cytoplasm cytokinesis proteins.

Inhibitors of FtsZ have already been shown to prevent FtsZ polymerization and bacterial cell division. The molecules bind to the one of the two alternative sites of FtsZ (fig 1) at the end terminal GTP binding site or at the C terminal inter domain cleft. Compounds targeting the highly conserved GTP binding site mimic the natural substrate of

the enzyme and might have potential advantage for developing broad spectrum anti bacterial activity. [20]

FtsZ monomers assemble into a Z ring during bacterial cell division. GTP binding and hydrolysis regulates assembly and organization of FtsZ into ring like structure. (this process is modulated by interaction of n terminal binding domain of Ft Sz monomer to c terminal GTPase activity domain (t72) on the adjacent Ftsz monomer)

Subsequently FtsZ recruits other proteins to form a cell division complex known as divisome. After full assembly of divisome bacterial cell division takes place by co ordinate constriction and splitting of daughter cells hence a drug which targets FtsZ proteins and GTP binding site prevents polymerization of FtsZ leading to failure of bacterial cell division.[20]

Recent evidence confirms that, berberine inhibits FtsZ which is an important protein regulating bacterial cell division by molecular docking stimulation. It was shown that berberine binds to C terminal inter domain cleft of FtsZ projecting the 9 Methoxy group towards the outside of cavity These docking results have initiated the study of 9 Phenoxy alkyl barberine derivatives to study their antimicrobial effect. [20]

Antimicrobial activity of 9 Phenoxy alkyl substituted berebrine derivatives (1-7)

9 Phenoxy alkyl substituted derivatives of berberine have shown potent antimicrobial activity against gram positive bacterial strains such as Ampicillin and Methicillin resistant s.aureus . They also have broader spectrum activity than parent compound berberine.[20]

The newer berberine derivatives target FtsZ bacterial proteins. These compounds were potent inhibitors of GTPase activity of FtsZ and they also inhibited FtsZ polymerization in a dose dependent manner. This was explained on basis of binding of berberine derivative into the inter domain cleft which interferes the GTPase activity of FtsZ. This in turn disturbs (destabilization) formation FtsZ polymers. [20]

Berberine derivative 1-6 (having Phenoxy alkyl group at C9 position) have not only exhibited potent action against gram negative bacteria but also shown improved broad spectrum antibacterial activity than parent compound. Compounds 1-6 suppressed both drug sensitive and resistant strains and also Vancomycin sensitive and resistant strains of E. faecium. Gram negative organisms like E. coli and K. pneumonia were also suppressed.[20]

Compound 7 without 9 Phenoxy group was less potent than compound 1-6 suggesting that aromatic ring play an important role in antibacterial activity. Compound 2 and 5 have shown stronger antimicrobial activity than Ampicillin and Vancomycin even against antibiotic resistant S. Aureus and E. faecium which suggest that the berberine derivatives are not affected by common mechanisms of bacterial antibiotic resistance. This proves that berberine itself or its derivatives are potential antimicrobial drugs. [20]

Anti fungal

Candida species are now the fourth most common organism recovered from the blood of hospitalized patient [22]. The available Antifungals though effective are known for their high toxicity. It has been observed that Berberine has weak activity against C. albicans and C. glabrata [23,24]. Hence various studies have been conducted to assess their adjunctive role when combined along with the various antifungals (azoles and amphotericin).

As a single agent, berberine showed weak antifungal activity against the fluconazole-resistant isolates tested, when the activity of the two agents in combination was evaluated, the MIC80 value of berberine was markedly decreased, and the MIC80 value of fluconazole was also decreased. [25]

Prolonged administration of antifungals in immunocompromised patients leads to formation of azole resistant strains making the eradication of infection difficult. Azole-resistant clinical isolates over express multi-drug efflux transporters including ABC transporters v or major facilitator super family (MFS) transporters [26]. Suppressing the activity of these fungal ABC transporters with small molecule multi-drug efflux pump inhibitors could reduce the drug resistance of these pathogenic fungi and therefore help to increase the efficacy of antifungal chemotherapy with triazoles.

Amphipathic cations are the preferred substrates of most multidrug resistance pumps [27]. As mentioned earlier berberines are amphipathic cations. Several studies have shown that plants of the genus Berberis (Berberis repens, B. aquifolium and B. fremontii) producing berberine synthesise two substances, the flavonolignan 50-MHC-D and the porphyrin pheophorbide a, which have no antibacterial activity but have an inhibiting property against MDR efflux pumps found so far in Staphylococcus aureus (allowing berberine to carry out its activity) [21, 28,29]

Study was conducted by introducing various aromatic groups in 13-C of berberine and berberrubine, a series of 13-(substituted benzyl) berberine and berberrubine derivatives to assess the antifungal activities against various human pathogenic fungi. The synthesized compounds exhibited more potent antifungal activities than berberine and berberrubine. Among them, 13-(4-isopropyl benzyl) berberine exerted the most potent antifungal activities against candida species and a 4-fold stronger activity than 13-(4-isopropyl benzyl) berberrubine synthesized by pyrolysis of the compound.[30]

Similarly incorporation of berberine hydrochloride in the trial denture cleansers have shown to reduce the adherence of the cells to the dentures of patients.[31]

Based on the above mentioned studies berberine can be an attractive perspective for managemement of candidiasis as the effective berberine concentration in vitro can be achieved in vivo.

Antiprotozoal

Berberine extracts and salts has demonstrated growth inhibition *of Giardia lamblia, Entamoeba histolytica, Trichomonas vaginalis*, and *Leishmania donovani*, with crude extracts being most effective. [32-34]

Clinical trials have shown that berberine administration improved gastrointestinal symptoms and resulted in reduction in Giardia-positive stools. Berberine was as effective as metronidazole but that too at half the dose. [35]

Both *in vivo* and *in vitro* studies of berberine's effects on *E. histolytica* indicated berberine sulfate was rapidly amoebicidal and caused encystation, degeneration, and eventual lysis of the trophozoite forms.[36]

Berberine sulfate rapidly inhibited the growth of *Trichomonas vaginalis* via formation of large autophagic vacuoles that eventually result in the lysis of the trophozoite forms. *In vitro* results indicated that berberine inhibited multiplication, respiration, and macromolecular biosynthesis of amastigote forms of the parasite and interfered with the nuclear DNA of the promastigote form, and inhibited organism maturation.[32,33]

In a study, a new semi-synthetic berberine analogue, 5,6-didehydro-8,8-diethyl-13-oxodihydroberberine chloride, showed nanomolar level potency against in vitro models of Leishmaniasis, Malaria, and Trypanosomiasis as well as activity in an in vivo visceral Leishmaniasis model.[37]

A study conducted to assess the role of berberine in Schistosoma mansoni-induced hepatic injury in mice it was observed that use of berberine for ten days had a definite antischistosomal effect and has a antifibrotic role and also reduces the S mansoni induced oxidative stress due to its antioxidant property. K. C singhal has proved antihelminthic action of berberine hydrochloride against syphecia ovevalata in mice.[38]

Berberine and its analogues can be used to treat various protozoal infections but there is definitive need for further studies.

Anti viral

Along with its antibacterial and antiprotozoal effect berberine has also been investigated quite extensively for its possible antiviral effect. Various studies have been conducted to assess its antiviral effects against specific viruses like influenza, cytomegalovirus and herpes simplex virus.

Study was conducted by Wu Y et al to assess the anti influenza effect of berberine. In vitro anti-influenza virus assays were performed by cytopathogenic effect and neuraminidase assays in Madin Darby canine kidney cells. In vivo anti-influenza virus assays were performed on the viral pneumonia model of mice. Berberine showed inhibitory effects on cytopathogenic effects and neuraminidase activity of virus, with the therapeutic index 9.69. In vivo, berberine decreased mice mortality from 90% to 55%, reduced virus titers in the lungs on day 2 postinfection. The production of nitric oxide (NO) and inducible nitric oxide synthetase (iNOS) were repressed along with inhibition of transcription and expression of TNF-α and Monocyte specific chemoattractant molecule (MCP-1). [39]

Berberine exhibited antiviral effects on the influenza virus both in vitro and in vivo. The possible therapeutic mechanism of berberine on influenza-induced viral pneumonia might be the inhibition of the virus infection, as well as improving the pathogenic changes by repressing inflammatory substances release.[39]

In a study conducted to test whether the isoquinoline alkaloid berberine can inhibit the growth of influenza A. Studies of the mechanism underlying this effect suggested that berberine acts post-translationally to inhibit virus protein trafficking/maturation which in turn inhibits virus growth. Berberine was also evaluated for its ability to inhibit production of TNF-α and PGE_2 from A/PR/8/34 infected-RAW 264.7 cells. These studies revealed strong inhibition of production of both mediators and suggest that this effect is distinct from the anti-viral effect. Finally study was conducted to evaluate, whether berberine containing ethanol extracts of goldenseal also inhibit the growth of influenza A and production of inflammatory mediators. They found strong effectiveness at high concentrations, although upon dilution extracts were somewhat less effective than purified berberine. Taken together, their results suggest that berberine may indeed be useful for the treatment of infections with influenza A[40].

Berberine is an alkaloid extracted from *Coptidis rhizome.* Among the individual herbal components of a Chinese herb medicine, Ching-Wei-San, *Coptidis Rhizoma* has the most potent antimicrobial activity. Study was conducted to explore the potential use of Ching-Wei-San against herpes simplex virus (HSV) infection, the cytotoxicity, anti-HSV-1 and anti-HSV-2 activity in Vero cells were assayed. The selectivity index of berberine was about 1.2–1.5 times higher than that of Coptidis rhizome extract and Ching-Wei-San. Moreover, the antiviral activities corresponded to the content of berberine in the aqueous solution. Berberine may interfere with the viral replication cycle after virus penetration and no later than the viral DNA synthesis step, and its activities were not affected by the preparation processes. Berberine, the natural plants that contain this component, including Coptidis rhizome,and Ching-Wei-San have all shown anti-HSV effects. [41]

Berberine chloride and the structurally related compounds were assessed for the anti-human cytomegalovirus (HCMV) activity using the plaque assay. The anti-HCMV activity of berberine was equivalent to that of Ganciclovir (GCV). The mechanism of action by which berberine inhibits the replication of HCMV is presumed to be different from that of GCV; berberine would interfere with intracellular events after virus penetration into the host cells and before viral DNA synthesis[42]

CONCLUSION

Berberine has been widely used in traditional Chinese and ayurvedic medicine. It has shown potential as an effective antimicrobial agent provided the problems of low solubility and bioavailability can be dealt with. There is a definite need of conducting planned randomized trials to further prove its efficacy as an antimicrobial agent and also to discount any possible drawbacks.

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