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A bird's eye view on a therapeutically 'wonder molecule': Berberine

Parth Patel*

H. K. College of Pharmacy, Jogeshwari West, Mumbai 400102, Maharashtra, India

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ABSTRACT

Berberine is a quaternary ammonium salt and naturally occurring benzylisoquinoline alkaloid, present in numerous medicinal herbs' roots and stem bark as an active constituent, especially in the genus Berberis. It contains many pharmacological properties such as antioxidant, antiviral, antidiabetic, antidepressant, antidiarrheal, antibacterial any many more. Since nature is the best healer, it has been traditionally used in Ayurvedic and Chinese medicine to mitigate several disease conditions. Besides the beneficial effects of berberine, some drawbacks such as its poor aqueous solubility and low oral bioavailability hinder its applications. Although it has been used for dietary supplements, despite its vast potential to evolve as a drug candidate, there are no approved pure berberine formulations available in market for any particular disease. Therefore, this review provides an overview to the reader by incorporating recent studies on berberine's sources, extraction techniques, chemistry, different Nano carriers and bioavailability enhancers for enhancing bioavailability, versatile applications, toxicological aspects and recent patents along with future perspectives. The accumulated evidence may broaden the horizon of drug designers, scientists, academicians, and researchers on berberine and help design and develop effective berberine formulations on a large scale for treating several diseases.

1. Introduction

Nature's one of the best gifts to mankind is the 'Amazon forest', which is undoubtedly the most excellent natural repository of plant diversity and the most diverse ecosystem on the planet. Plant natural products (PNPs) have been an inspiration for a substantial proportion of commercial pharmaceutical products for animals, humans, and crops over the past few decades. The taxonomic, structural and molecular diversities, high safety, availability, accessibility and low cost of natural products provide significant advantages for driving pharmaceutical discovery (Chen et al., 2020). In recent decades, the pharmacological properties of natural herbs have been more apparent, and, as a result, herbal medicine has opened new horizons for the healthcare sector. Medicinal herbs have been used and profoundly valued since ancient times as a folk medicinal cure for preventing and treating various diseases (Samadi et al., 2020).

Alkaloids are one of these PNPs that are highlighted and promising compounds owing to their high structural diversity and a vast array of biological activities, including anti-cancer, anti-viral, antimicrobial, antifungal, antispasmodic and acetyl cholinesterase (AChE) inhibition (de Lima et al., 2019). Berberine, a nonbasic, crystal yellow coloured, quaternary benzylisoquinoline alkaloid, belonging to the class of protoberberine alkaloids, is found to have a 3000 years long history of usage as an Ayurvedic and Chinese medicine for its potent anti-bacterial (Peng et al., 2015), anti-cancer (Han et al., 2020), antidiabetic (Zhou et al., 2019), anti-obesity (Asbaghi et al., 2020), neuroprotective (Chen et al., 2020), anti-diarrheal (Joshi et al., 2011), anti-inflammatory(Y. B. (Shen et al., 2010), anti-depressant (Sun et al., 2014) and hepatoprotective activity (Kumar et al., 2015, Zhao et al., 2018). Berberine is hydrophilic and undergoes extensive metabolism; thus, the bioavailability upon oral administration is extremely low. However, several Nano strategies have been implemented by researchers to increase its bioavailability. Moreover, certain drugs, when co-administered, significantly increases its absorption. In clinical practice, berberine is used to treat several disorders such as cancer, type-2 diabetes mellitus, Alzheimer disease etc. Also it has been used as a chemical marker for

Abbreviations: BBR-BS20-NCs, Berberine- Brij-S20- nanocrystal; RSD, Relative Standard Deviation; MCF-7, Michigan Cancer Foundation-7; IC_{50}, Half-maximal inhibitory concentration; MAO-B, Monoamine oxidase –B; PDE4, Phosphodiesterase 4; GLP, Glucagon-like peptide; Nrf2/ HO-1, Nuclear factor erythoid 2- related factor-2/ Nuclear heme oxygenase-1; Pl3k, phosphoinositide 3-kinase; Akt (or PKB), Protein kinase B; UCP2, Uncoupling protein 2; Bcl-2, B-cell lymphoma 2; NADPH, Nicotinamide adenine dinucleotide phosphate; NF-κB, nuclear factor kappa light chain enhancer of activated B cells; HIV-1, Human Immunodeficiency Virus Type 1; MCP-1/CCL2, Monocyte chemoattractant protein-1/ chemokine (C-C motif) ligand 2; ACE2, Angiotensin-Converting Enzyme 2; TMPSS2, Transmembrane protease serine 2; TGF-β1, Transforming growth factor beta 1; TLR4, Toll-like receptor 4; LD_{50}, Lethal dose; HED, Human equivalent dose; LOAEL, Lowest Observed Adverse Effect Level.

* Corresponding author.
E-mail address: parth.patel@hkcp.edu.in

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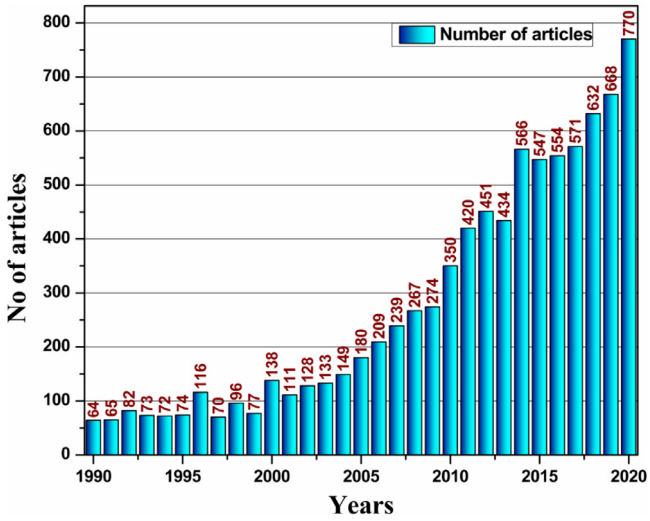

Fig. 1. Number of publications in Web of Science Core Collection database on Berberine (Search date: February 4, 2021).

quality control of many prescriptions such as Huanglian-Jie-Du-Tang, Gegen- Huangqin-Huanglian-Tang and Zuo-Jin-Wan, which have been used in clinical treatment since decades (Wang et al., 2017). Nowadays, berberine has been receiving a significant amount of attention by researchers due to its excellent biological activities as there is growing number of studies focusing on berberine. To justify, by glancing on **Fig. 1**, it can evidently be seen that berberine has been in the limelight since last few years considering the increasing number of publications, thus serving as a source of inspiration to write this article. Therefore this review is designed with an aim to summarize various aspects of berberine such as its source, chemistry, bioavailability, toxicological aspects, applications and patents.

2. Sources

The Berberis genus of more than 500 species belongs to the Berberidaceae family. Berberine occurs as an active constituent in the root, rhizome and stem bark of many therapeutically important plants, including *Hydrastis canadensis* (goldenseal), *Berberis aquifolium* (Oregon grape), *Berberis vulgaris* (barberry), *Coptis chinensis* (Coptis or goldenthread), and an Indian species *Berberis aristata* (Tree turmeric). Among these, berberine is predominantly found in various barberry species and goldenseal species native to Asia and America. *Berberis aristata*, (family: Berberidaceae) known by various popular names such as Chitra, Daruhaldh, Daruharidra, Kashmal, is a spiny shrub of up to 3 meters, growing at an altitude of 2000 to 3000 meters and is widely distributed in the regions of Himalaya and Nilgiri hills in Southern provinces of India. Its active constituents include berberine, berbamine and palmatine (Kumar et al., 2015). Additionally, berberine is also found in plants of families Papaveraceae and Ranunculaceae. In a traditional chinese herb, *Rhizoma coptidis* (Huang Lian), researchers have reported berberine as a major active component in the range of 5.2-7.7%. (Singh and Sharma, 2018)

3. Physical and chemical properties

Chevallier and Pelletan first isolated berberine in the year 1826 from *Xanthoxylon cava*. Berberine is a yellow coloured, isoquinoline alkaloid, crystalline, stable, quaternary amine and has a molecular formula of $C_{20}H_{18}NO_4^+$(16,17-dimethoxy-5,7-dioxa-13 azoniapentacyclo [11.8.0.02,10.04,8.015,20] henicosa-1(13),2,4 (8),9,14,16,18,20-octaene), and a molecular mass of 336.4 g/mol (**Fig. 2**). It is readily soluble in hot ethanol, slowly dissolves in water. Moreover, it is not much soluble in organic solvents like chloroform and benzene (Xu et al., 2021). Berberine is a permanently charged compound. The extent of berberine's water solubility depends primarily upon buffer and temperature and is independent on pH; due to the absence of any ionisable group. It has been observed by Battu *et al.* in their study that, as temperature increases, the solubility of berberine chloride in water slightly increases (solubility at 25 is 5.27 ± 0.29, and at 37°C solubility is 8.50 ± 0.40 mM,). Furthermore, their study also revealed that berberine chloride showed maximum solubility in phosphate buffer (4.05 ± 0.09mM at 25°C and 9.69 ± 0.37 mM 37°C) (Battu et al., 2010). Three tautomeric forms of berberine exist; the structure containing quaternary nitrogen forms salts with mineral acids such as HCI and H_2SO_4 by removing one water molecule are correctly classified as berberinium compounds (Hahn and Ciak, 1975). According to the literature, there are two different pKa values reported, 15.7 (Preininger and Santavy, 1969) and 2.47 (Roja et al., 2005), respectively indicating contradiction in berberine's acid/base character. At physiological pH, it has been found that berberine is a positively charged moiety because of the presence of iminium cation ($C{=}N^+$), which is polar in nature, and the absence of acceptor or proton donor group (Spinozzi et al., 2014).

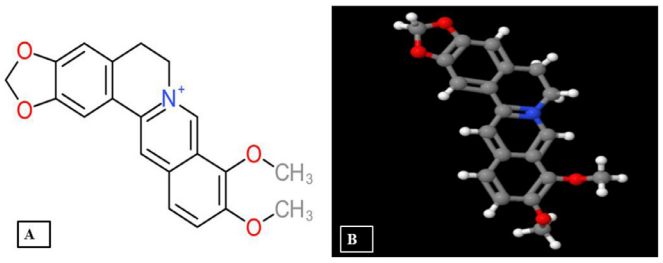

Fig. 2. Chemical structure of berberine a) 2D, b)3D (Source: ChemSpider® Database).

4. Extraction techniques

Berberine is one of the widely distributed alkaloids of its class. The principles behind the extraction techniques consist of interconversion reaction between the protoberberine salt and the base. The salts are water-soluble, stable in acidic and neutral media, while the base is organically solvent-soluble. Thus, the protoberberine salts are converted into their specific bases during the extraction process and further extracted into the organic solvents (Neag et al., 2018). As Berberine is sensitive to heat and light, exposing it to higher temperature and light can result in degradation and affect the yield. Even though conventional methods are widely used in berberine extraction, many other methods have been developed lately (**Fig. 3**). This led to improved extraction efficiency, a decreased extraction time and solvents' volumes used in the extraction (Neag et al., 2018). For instance, Ben *et al*, extracted berberine from rhizome of Coptis chinensis Franch by using Supercritical fluid technique. The highest yield was obtained after 3 hours by extracting with 1,2-propanediol-modified supercritical carbon dioxide. (Liu et al., 2006). In another study, berberine was extracted by Pressurized hot water extraction (PHWE) method (temp 95-140°C, pressure: 10-20bar, time-40 min). Extraction by PHWE was found to give efficiency comparable to soxhlet extraction (Ong and Len, 2003). Conventional techniques are also employed, such as percolation (Mahmoudvand et al., 2014), maceration, soxhlet, cold or hot continuous extraction by using solvents such as ethanol, methanol, petroleum ether etc. Extraction temperature and choice of solvent play a crucial role in berberine's extraction. It has been observed that aqueous or acidified ethanol or ethanol are generally used for extraction (Neag et al., 2018). In another novel technique, berberine was extracted from *Cortex Phellodendri* using Ultrahigh Pressure Extraction (UPE). The method offers several advantages such as less time consumption, high yield, reduced extracting duration

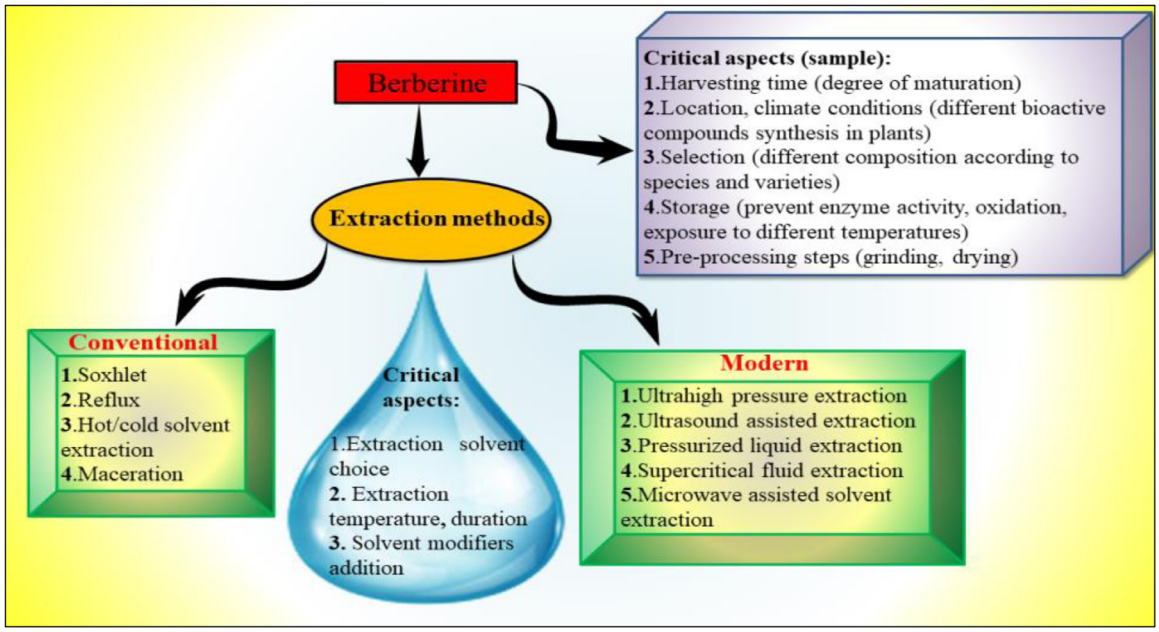

Fig. 3. Schematic representation of different methods used to extract berberine. (Neag et al., 2018).

and low extracting temperature. It was found that efficiency of berberine was 7.70mg/g, which was comparatively higher than conventional extractions (Guoping Liao et al., 2012) .Table 1 summarizes recent studies on extraction of berberine by various techniques.

5. Analytical techniques for detecting berberine

Since berberine is easily available, it has been widely used in traditional medicine and the pharmaceutical industry. Determination of bioactive content in any pharmaceutical or biological preparation is essential to ensure safety and efficacy (Feng et al., 2019).In case of berberine, analytical methods are used to evaluate the quality of drug preparations, help make quality standards, and analyse the absorption, distribution, and metabolism of berberine. Therefore, to achieve reasonable control and administration of berberine in food and other preparations, it becomes necessary to focus on qualitative and quantitative analytical studies for determining berberine in real samples, as they equally play a significant role in designing, developing and marketing a pharmaceutical formulation (Fei and Rui, 2011).To date, there are plethora of analytical techniques employed to detect berberine including Resonance light scattering spectrometry (RLS) (Liu et al., 2002), Thin Layer Chromatography (TLC) (Lu et al., 2015; More et al., 2017; Raj, 2020), Capillary Electrophoresis (CE) (Uzaşçı and Erim, 2014; Liu et al., 2006); (Sun and Tseng, 2004), High Performance Liquid chromatography (HPLC) (Liu et al., 2021; Qi et al., 2018), chemiluminescence (Biparva et al., 2016), Ultra Performance Liquid Chromatography (UPLC) (Liang et al., 2020; Wang et al., 2016),electrochemical analysis (Wang et al., 2020; Liu and Chen, 2012), colorimetric detection (Gao et al., 2021; Hu et al., 2017), mass spectrophotometry (MS) (as GC-MS, LC-MS) (Liao et al., 2019) etc (Wang et al., 2019). **Fig. 4** illustrates the main methods used for the detection of berberine currently. Traditionally, researchers have been using these methods to detect and quantify berberine. However, each of these methods has its distinct advantages and drawbacks that are highlighted in **table 2**. Therefore, considering the drawbacks of conventional analytical techniques, researchers are developing novel analytical techniques to determine berberine. For instance, Liu and co-workers, in their recent study, have demonstrated that cationic carbon quantum dots (CQDs) can be used to visually detect berberine in human blood serums. The ratiometric fluorescent probe has high sensitivity towards alkaloids and metal ions, photochemical stability (60 min), and pH stability (from 6.0 to 8.0), with the detection range from 0 to 200 μM. As compared to traditional techniques, this method had an extra edge in terms of better selectivity, high sensitivity, easy operation, and was inexpensive which could be widely utilized as fluorescent nanoprobes to detect various compounds quantificationally (Liu et al., 2021). Similarly, another study had also determined berberine by using water soluble thioglycolic acid-capped CdTe quantum dots (TGA-CdTe QDs) as probes and reported that the relative fluorescence intensity was linearly proportional to the concentration of berberine between 2.5×10^{-8} and 8.0×10^{-6} molL^{-1} (Cao et al., 2010). Apart from this, nanoparticles have also used to detect berberine. For eg, Liu *et al.*, determined berberine hydrochloride using simple, sensitive and green spectro fluorimetric method with silica nanoparticles (SiO2NPs) as a probe. Their results revealed that the linear range of the method was from 2.0–50.0 mgL^{-1} with a detection limit of 0.73 mgL^{-1} (Liu et al., 2018). **Table 3** summarizes recent analytical studies in berberine determination.

6. Berberine bioavailability

Various parameters affect the oral bioavailability of berberine such as dosage forms (rate of dissolution and degree of dispersion), physicochemical properties (permeability, stability in the environment of GI tract, solubility) and physiological aspects (metabolism in gut wall and liver). Although there are contradictory values of bioavailability of berberine reported, 0.36% (Liu et al., 2010) and 0.68% (Chen et al., 2011), however, the absolute bioavailability is less than 1%. (Liu et al., 2016). Although the exact mechanism of low bioavailability of berberine in not completely understood yet, however, there are some studies which have proposed some possible reasons accounting for its low bioavailability. Firstly, considering the structural aspects, berberine is a hydrophilic compound which is the primary reason that limits its absorption. The strong hydrophilicity behaviour of this compound is contributed by various parameters such as octanol-water partition coefficient (lipophilicity) (Log P-value: -1.51), topological Polar Surface Area (tPSA value: 40.8 A°), percent Hydrophilic Surface Area (HSA value: 25.97%) (Battu et al., 2010, Fernandes and Gattass, 2009). Due to berberine's lipophobicity, crossing the plasma membrane of intestinal cells is hindered. Secondly, there was extensive intestinal first-pass elimination observed. Finally, ATP-binding cassette (ABC) trans-

Table 1
Summary of recent studies on extraction of berberine by various techniques.

Sr.No	Sample	Extraction method	Description/Methodology	Observations	Reference
1	Roots of *Berberis lycium*	Soxhlet extraction (SE)	Central composite design-response surface methodology (CCD-RSM) were used to investigate the extraction variables.	The highest yield (13.39%) was obtained by maintaining optimal extraction conditions, i.e., extraction time (7.28 hrs), ethyl alcohol (52.21%) and solvent to sample ratio (21.78 v/w)	(Katare et al., 2020)
2	Fruits, leaves, and stems of *B.integerrima and B.thunbergii*	Maceration	CCD-RSM were used in this study to investigate the extraction variables	The best condition for extraction of berberine by maceration obtained with ethanol 90% in 3.36 h (141.6 min) at 70° C.	(Sarraf et al., 2020)
3	From *Phellodendri amurensis* cortex	Ultrasound-assisted extraction (USE)	Green solvents such as Deep eutectic solvents (DES) were used for extraction. DES-1: (choline chloride + citric acid)	Predicted data concentration: 9.60mg/g, experimental data concentration: 9.64mg/g of berberine; Optimal extraction parameters: concentration of Deep eutectic solvent (DES-1): 30 wt%, time: 30min, Temperature: 60°C.	(Li et al., 2020)
4	Roots of *Berberis Aristata*	Microwave-assisted subcritical water extraction (MASWE)	Harmony Search Algorithm (HSA) was used to study the effect of five subcritical parameters on the yield of Berberine	Experimental data concentration: 223.82 µg/ml, predicted data concentration, 214.854 µg/ml of berberine. Optimal extraction parameters: temperature 170°C, the particle size of 0.65 mm, time of 70 mins, solvent/meal ratio of 12 and maximum of three repetitions.	(Kumar Manikyam et al., 2017)
5	From *Phellodendri amurensis* Cortex	Ultrahigh pressure Extraction (UPE)	Orthogonal design was applied to evaluate the effects of four independent factors (extraction pressure, extraction temperature, liquid/solid ratio and ethanol concentration)	The optimal conditions of UPE were obtained at an extraction pressure of 400 MPa, extraction temperature of 40 C, extraction time of 4 min, a liquid/solid ratio of 30: 1 and an ethanol concentration of 50%. Yield: 1.93 ±0.06%.	(Liu et al., 2013)

Table 2
Advantages and disadvantages of commonly used analytical techniques.

Sr.no	Technique	Advantages	Disadvantages
1	HPLC	-Automatic operation -High detection sensitivity -Good selectivity -High separation efficiency -Quantitative sample recovery	-High analysis cost -Detection limit is not low enough -Less separation efficiency than capillary gas chromatography (GC
2	TLC	-Low cost -Easy to perform -Good accuracy and stability -No instrumentation required -Easy to display colour -Wider choice of mobile phase	-Chances of evaporation of mobile phase -Limited sample size -Possibility of occurring edge effect. -As it operates in an open system, factors such as humidity and temperature can affect the results.
3	HPTLC	-Shorter analysis time -Less consumption of solvent / mobile phase -Parallel separation of multiple samples with minimal time -Higher separation efficiency/resolution	-Too costly -Maintenance of instrument is difficult -Requires a trained and experienced technician to operate the instrument.
4	CE	-Easy and predictable selectivity -Easily couple with MS -Rapid separation -High separation efficiency -faster than HPLC	-Reproducibility issues -Not suitable for larger proteins (>20kD).
5	LC-MS	-Simultaneous multi-analyte analysis -No derivatization needed -Wide application range -High sensitivity -Strong separation ability - Suitable for analysis of relatively polar compounds with low, moderate or high molecular weights	-Expensive -Not portable - Matrix effects in LC-MS may alter the stability and reproducibility.
6	GC-MS	-Suitable for analysis of low molecular weight hydrophobic compounds. -Volatile compounds can be directly analysed - High qualitative reliability -Efficient quantitative analysis -Accurate structural analysis.	-Not suitable for non-volatile and thermo-unstable compounds -Requires derivatization. -Derivatisation can mask the result.

Table 3
Summary of recent qualitative and quantitative analytical studies on berberine.

Sr no	Detected components	Sample preparation	Analytical method and description	Results	Validation	Remarks	Reference
1	Berberine HCL in bulk and synthetic mixture	Extracted with methanol and sonicated for 15 min	UV absorbance (λ_{max} = 265nm)	Drug response with respect to absorbance was linear over the concentration range 10-60 μg/ml for berberine; Percent recovery:-99.48%;	% R. S. D. values for intra-day, and the inter-day precision study- <2.0%; LOD- 0.86 µg/ml; LOQ- 2.60 µg/ml	Simple and accurate method, no interference of formulation excipients in the estimation of berberine	(Patel et al., 2020)
2	Berberine (from a polyherbal formulation *Pushyanuga* Churna)	Extracted with methanol on a vertical test tube rotator (25 rpm)	**1) HPLC:** Column- Cosmosil C8 column (150 × 4.6 mm, 5.0 µm); MP- 0.2% trifluoroacetic acid in water–ACN (70+30, v/v); flowrate- 1 mL/min **2)HPTLC:** HPTLC plates- silica gel $60F_{254}$; MP- toluene–ethyl acetate–methanol–FA (6+6+2+1, v/v/v/v)	**1)HPLC**:Optimum resolution, with R_t:8.061 ± 0.022 min; detection was made at 348 nm using a PDAD; percent recover:94.78–97.89% **2)HPTLC:** Rf value- 0.24; Percent recovery:95.25–97.65%	**1)HPLC:** Linearity range:0.05–15.0 μg/mL; LOD-0.01 μg/mL; LOQ:0.05 μg/mL; **2) HPTLC:** Linearity range:0.1–15.0 μg/mL; LOD-0.05 μg/mL; LOQ-0.1 μg/mL; **By HPLC and HPTLC:** %R. S. D. values for intra-day, and the inter-day precision study- <2.0%;	Applicable for simultaneous estimation of biomarkers from plants, formulations, and other biological matrixes, used for the identification of proper ingredients and to prevent adulteration.	(Shailajan et al., 2019)
3	Berberine and its nine metabolites(in rat plasma)	Acetonitrile was added to rat plasma containing berberine and was centrifuged	UHPLC-Q-TOF-MS. (C18 RRHD column (2.1 × 50 mm, 1.8 µm), T=30°C, MP=Solvent A (0.1% of FA in water and 10 mM ammonium acetate) + Solvent B (ACN), flow rate- 0.3 mL/min	The nine metabolites, M1-M9, were eluted at 2.79, 2.78, 2.23, 2.81, 3.72, 2.85, 3.82, 3.90 and 5.43 min respectively; several new metabolic pathways and three metabolites in rat plasma are reported for the first time	-	This rapid, sensitive and high resolution UHPLC-Q-TOF-MS method could detect extremely low concentrations of berberine metabolites.	(Xu et al., 2019)
4	Berberine (in bulk and pharmaceutical dosage form)	Mother tincture was prepared of marketed formulation and was dissolved in MP followed by sonication.	RP-HPLC: Column: Kromosil (250 × 4.6mm, 5μ); MP-0.1%tri fluoro acetic acid:acetonitrile (70:30v/v); flow rate- 1.0 ml/min	Retention time (R_t)- 5.003 min, retention time was decreased and run time was decreased; Percent recovery: 92-98%; well-shaped peaks; no interference from placebo with sample peak; assay percentages of berberine present in the samples were found to be 99.61% and 109.87%	Linearity range: 2-12µg/ml; LOD: 0.488 µg/ml; LOQ: 1.478 µg/ml; %RSD value for inter and intra day was found to be less than 2%	Simple and economical that can be adopted in regular Quality control test in industries.	(Padmavathi et al., 2019)
5	Berberine (in commercial capsule preparations available in the U.S market)	Methanolic extract was prepared, followed by sonication and centrifugation	UHPLC-MS/MS: Column: Luna Omega C18 column (100 × 2.1 mm, 1.6 µm, 100 ˚A); MP-A: 0.1% FA in H_2O; MP-B: 0.1% FA in ACN; flow rate: 0.4 mL/min; internal standard: DPG	The average berberine content across the product was found to be 75% ± 25% of the product label claim.	-	-	(Funk et al., 2018)
6	Berberine hydrochloride	HPTS and berberine stock solutions were prepared and stored at 4°C, followed by dilutions at low concentrations.	HPTS probe (fluorimetric sensing)	On optimization, it was found that upon the addition of 6.0 μM berberine to 1.0 μM HPTS in buffer at pH 7.4, fluorescence quenching equilibrium was achieved in 3 min; when the concentration of berberine ≥ 50 μM, quenching of green colour fluorescence was clearly observed by the naked eye; more efficient fluorescence quenching was observed at low temperatures; percent recovery: 93.7–106.2%	LOD- 1.24µM; linearity range-2–50 μM	Simple preparation, excellent water solubility, low cost, fast response, and high sensitivity and selectivity. This method can be used to detect berberine in real samples such as tablets and urine	(Guo et al., 2019)

Abbreviations: DPG: 1,3-diphenylguanidine, RSD: Relative Standard Deviation, LOD: Limit of Detection, LOQ: Limit of Quantitation, HPTS: 8-hydroxypyrene-1,3,6-trisulfonic acid, RP-HPLC: Reverse phase High Performance Liquid Chromatography, MP: Mobile Phase, FA: formic acid, ACN: Acetonitrile, PDAD: photodiode array detector

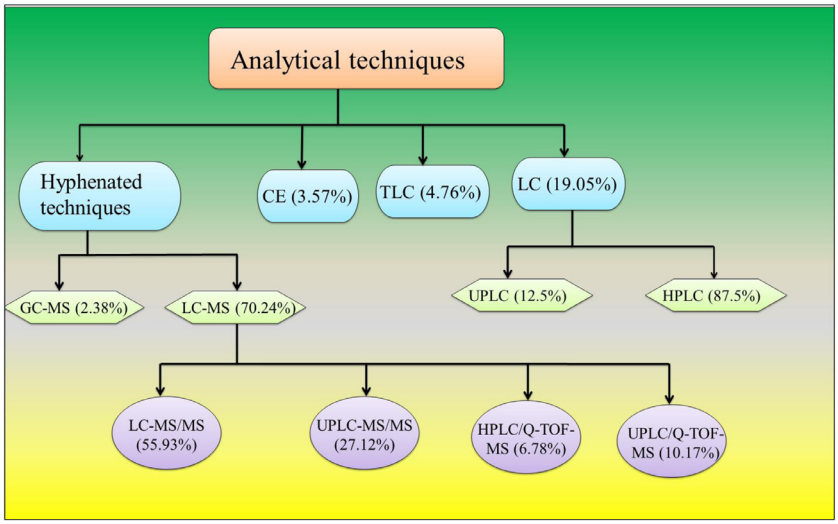

Fig. 4. Schematic representation of commonly analytical techniques used to detect berberine along with their percentage of usage.

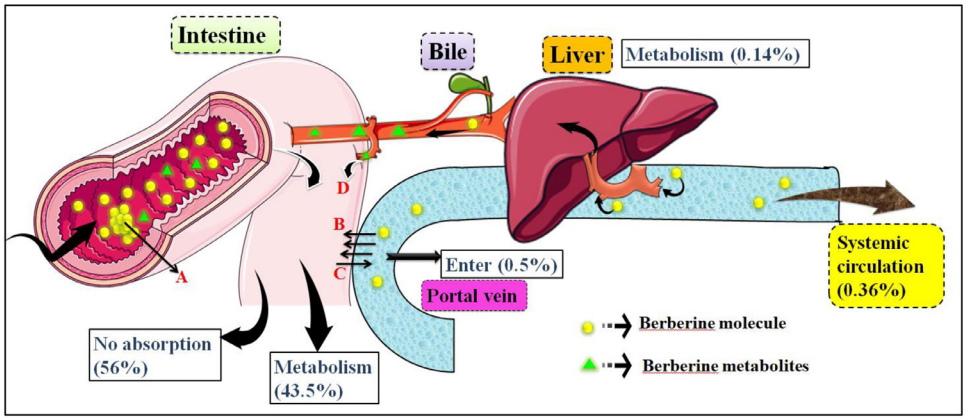

Fig. 5. Fate of berberine after oral administration (*in-vivo*): A) Due to self-aggregation, 56% of the drug is unabsorbed in the gastrointestinal tract (GIT); B) P-glycoprotein (P-gp) mediated efflux, C) Poor permeability, D) Hepatobiliary re-excretion. Out of the total administered dose, only 0.5% entered the portal vein; and 0.14% got metabolized in the liver (28% of the fraction). Therefore, absolute bioavailability is nearly 0.36% (Liu et al., 2016)

porters such as P-glycoproteins (P-gp) could interfere with absorption by directly effluxing the absorbed berberine back into the intestine. (Gan, 2016); (Liu et al., 2010). However, absorption is significantly increased when co-administered with P-glycoprotein inhibitors such as Silymarin from Milk Thistle. In addition, Sodium Caprate (a medium-chain fatty acid and an ester of Capric Acid) also enhances berberine absorption by widening the gaps between intestinal cells reversibly, thereby allowing passive diffusion (Preeti et al., 2015). **Fig. 5** represents the fate of berberine after oral administration. Researchers have used several nanoparticulate approaches to enhance bioavailability and solubility, such as solid-lipid nanoparticles, polymeric nanoparticles, magnetic silica-based nanoparticles, gold nanoparticles, micelles, liposomes and many more (Mirhadi et al., 2018). For instance, Fei *et al.*, developed novel self-assembled berberine loaded PEG–lipid–PLGA nanoparticles using Berberine–soybean phosphatidylcholine complex as liposolubility enhancer via solvent evaporation technique for enhancing oral bioavailability. They reported a significant increase in oral bioavailability (343%) compared to raw berberine (Yu et al., 2017). In another study, Xiong *et al.* developed a Brij-S20-modified nanocrystal formulation with an aim to improve intestinal absorption of berberine. Their findings revealed that the relative bioavailability of BBR-BS20-NCs to pure berberine was found to be 404.1% (Xiong et al., 2018). Several other recent nanoformulation studies of berberine are summarized in **table 4**. Based on various studies, it has been found that berberine enhances the bioavailability and activity of certain drugs. For example, Xin *et.al*; demonstrated that berberine increases oral bioavailability of cyclosporine A at a dose of 3mg/kg in healthy volunteers. This is possibly due to a decrease in metabolism in the intestine and liver through inhibition of CYP3A4(Xin et al., 2006); (Liu et al., 2006). Similarly, another study reported that berberine enhanced the bioavailability of digoxin and cyclosporine A in a dose-dependent manner in rats by inhibiting intestinal P-gp (Qiu et al., 2009). Berberine also enhances bactericidal effect of enrofloxacin *in-vitro,* suggesting its potential use in fish disease management in aquaculture (Zhang et al., 2010).

7. Chemistry

Berberine has a tetracyclic skeleton derived from a framework of benzyl tetrahydroisoquinoline incorporating an extra carbon atom supplied through the N-methyl group by S- adenosyl methionine (SAM). By an oxidative mechanism in which the N-methyl group gets oxidized to an iminium ion, the berberine bridge is readily rationalized, and the phenolic group cyclizes the aromatic ring. (Preeti et al., 2015). It is essential to characterize the excretion, pharmacokinetics profile of berberine, and its metabolites for clinical use. According to a study, 97 metabolites of berberine were reported in rats. (Wang et al., 2017). However, some major metabolites are extensively studied and quantified among all the metabolites, namely demethyleneberberine, berberrubine, jatrorrhizine, columbamine and thalfendine, and are formed by processes such as demethylation, demethylenation and hydrogenation. (Feng et al., 2021)

There are plethora of berberine derivatives that have been identified, mainly focusing on changes to the berberine positions C-12, C-8, N-7, C-13 and O-9. Modification of the 4-methyl group to a chloromethyl moiety improved the antifungal profile of azafluorenones and broadened it. By inhibiting MDR strains of *M.Tuberculosis*, the substitution of the n-decyl group at the 8-position could significantly improve anti-TB

Table 4
Summary of recent studies on different types of nanoformulations of berberine.

Sr.no	Type of Nano carrier/micro carrier	Method of preparation	Animal model	Particle size	Results	References.
1	Berberine nanoparticles	EPN and APSP	- *(in-vitro)*	EPN-71.53nm, APSP-102.63nm	Bioavailability of NP prepared by EPN method (D-76.8%, S-1.992mg/mL)was higher as compared to NP prepared by APSP method (D-74.1%, S-1.847mg/mL)	(Sahibzada et al., 2018)
2	Berberine nanoparticles	Antisolvent precipitation	Rabbits and Male Sprague-Dawley rats	102.62 ± 2.8 nm	The Nanoform of berberine was about 3.97 and 3.88 folds higher than that of the unprocessed berberine.	(Sahibzada et al., 2020)
3	Chitosan-coated alginate/gelatin Berberine loaded microspheres	W/O emulsification technique	Rats	368.2 μm	Berberine microspheres showed enhanced bioavailability(1.5-folds higher than commercial tablets) and drug release of 71.29% for 8 hours *in vitro,* and in-situ bio adhesion percentage was 91.23±8.2%	(Zhang and Liu, 2016)
4	Microspheres (SNAC used as an enhancer)	Solvent volatilization	Sprague–Dawley male rats	275.92 ± 14.02 µm	Berberine/SNAC-loaded microspheres significantly enhanced the bioavailability (14.14 times higher), EE- 65.52% ± 2.45%.	(Li and Zhu, 2020)
5	Selenium-coated nanostructured lipid carriers (SeNLCs)	Hot-melt dispersion/homogenization procedure followed by *in situ* reduction	Sprague-Dawley rats	160nm	The oral bioavailability of berberine-SeNLCs was found to be 6.63 times greater than that of berberine solution, EE-90%	(Yin et al., 2017)
6	Solid proliposomes (PL)	SEDS technique	Male rats	80nm	Prepared solid PL showed 22.47 times higher oral bioavailability than raw berberine, EE-90.3% ± 4.9%	(Jia et al., 2019)

Abbreviations: EPN-Evaporative Precipitation of Nanosuspension, APSP-anti-solvent precipitation with a syringe pump, S-Solubility, D-Dissolution, EE-Entrapment Efficiency, SNAC-(sodium *N*-[8-(2-hydroxybenzoyl) amino] caprylate, NP-Nanoparticles, SEDS- solution enhanced dispersion by supercritical CO_2.

activity. Upon addition of hydrophobic phenyl or alkyl groups at the C-8 position of berberine, it has been found to increase antimicrobial activity (Aswal et al., 2017). Berberine derivatives produced by reducing quaternary ammonium cation (=N^+=) at the N-7 position to corresponding tertiary amine shows improved anti-diabetic activity. High cytotoxicity was demonstrated against several cancer cell lines by the 9-*O*-octanylated berberine derivatives. Furthermore, base-modified berberine derivatives of C-12 Mannich showed enhanced anticancer and antioxidant activities (Wang et al., 2017; Tavares et al., 2014; Lizhen Wang et al., 2020; L. Wang et al., 2020; Wang et al., 2020). C-10 and C-11 positions of ring D play a significant role in exhibiting several therapeutic activities. For instance, Wang *et al.* synthesized several novel berberine based derivatives and investigated the hypoglycemic activity by attaching methoxy group (OMe) at different position on ring D. Their findings revealed that OMe group, when substituted at C-10 and C-11 position, exhibited better hypoglycemic activity (Percentage increase in glucose consumption: 16.76 ± 7.20%, Solubility: 11.8mg/mL) than substituents at other position (Wang et al., 2019) (**Fig. 6**). Interestingly, in another study, Yang and co-workers reported that by attaching OMe group at both C-10 and C-11 positions, it enhanced the activity in low-density-lipoprotein receptor (LDLR) expression, thereby making it a potent cholesterol-lowering agent (Wang et al., 2012, Yang et al., 2008). It has been revealed from investigation that methylenedioxyl group (Dioxolane ring) and quaternary ammonium unit are essential for anti-diabetic activity (Iranshahy et al., 2014); (Grycova et al., 2007); (Wang et al., 2020). Dioxolane ring has been reported to be critical for cholesterol-lowering activity from preliminary structure-activity relationships (SARs). The chemical modification sites are primarily focused on the C-9 and C-13 positions for the anticancer activity; whereas for antibacterial activity, the chemical modification sites are primarily focused on the dioxolane ring, C-8, and C-13 (Li et al., 2020); (Wang et al., 2019); (Xu et al., 2020, Yang et al., 2008). Presence of 6-12 carbon spacer between 9-*O* and terminal –CH_3 group was optimum for potent anti-tumor activity. Mistry *et al.* also synthesized berberine-indole conjugates and screened for antioxidant and anti-tumor potential. It was found that the introduction of indole at 9-*O* position of the berberine scaffold exerted a high therapeutic index as compared to berberine. Also, the presence of alkyl spacer between berberine and indole scaffolds was essential to obtain cytotoxic potency. Incorporating halogen atom at the C-5 position of the indole ring enhanced the anti-cancer potential. The synthesized conjugates showed excellent anti-tumor and cytotoxic activity against HeLa cell line and CaSki cells lines (Mistry et al., 2016). In another study, Zhou *et al.* synthesized novel 12-aryl berberine derivatives were and evaluated their inhibitory effects on hypoxia-inducible factor (HIF-) 1 transcription. They reported that attaching biphenyl group at C-12 of berberine produced the highest inhibitory activity against HIF-1 transcription with an IC_{50} value of 0.74 µM and also emerged to be the most potent cytotoxic derivative against MCF-7 cell lines displaying IC_{50} of 0.98 µM. However, the introduction of heteroaromatic ring at C-12 diminished HIF-transcription inhibitory activity (Zhou et al., 2017).

Olliek *et al.,* in their study, demonstrated that p-substituted aryl ring at C-12 possessed good anti-bacterial activity but displayed high toxicity. Later, it was observed that introducing phenyl rings carrying large substituents reduced toxicity as well as enhanced the antibacterial activity against gram-positive bacterial strains, including *Bacillus cereus, Staphylococcus aureus* , and *Streptococcus pyogenes* with minimal inhibitory concentration (MIC) values ranged between 3.12–6.25 μM, while the tested compounds were found to be inactive towards the gram-negative strains except for *H. pylori* and *V. alginolyticus.* Furthermore, multiple substitutions at phenyl ring increased anti-bacterial activity (Olleik et al., 2020). Wang and co-workers synthesized berberine derivatives and evaluated their anti-staphylococcal activity. Their results revealed that MIC values of most of the derivatives were found to be 0.78-25 µg/ml. They concluded that the replacement of 9-*O* with 9-N remarkably improved the antibacterial activity against *S. aureus* (Wang et al., 2020). A study revealed that the introduction of cin-

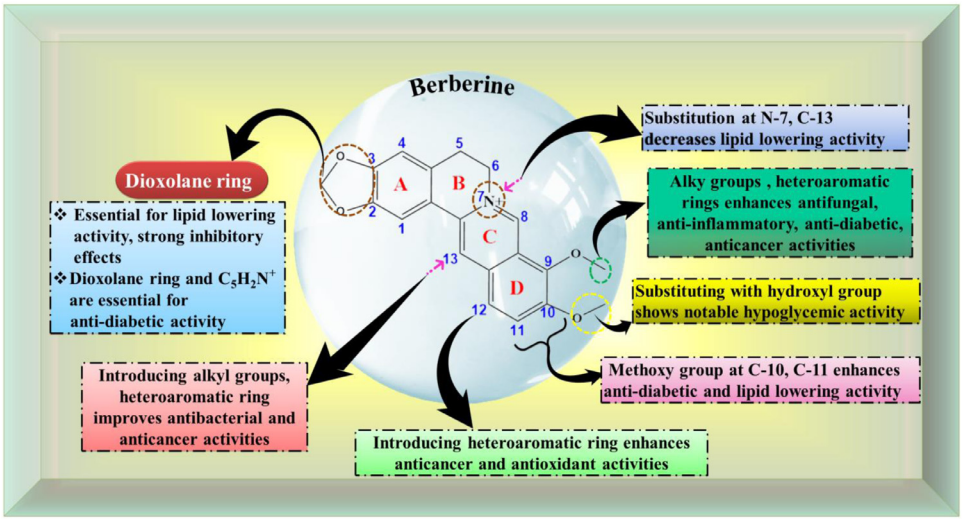

Fig. 6. Structural activity relationships (SARs) of berberine.

namic acid at the 9-*O* position showed excellent hypoglycemic effects (Zhang et al., 2016). Another study by a team of researchers have observed that glycosylation at 9-*O* position imparted potent hypoglycemic effects. Also, by incorporating triazole spacer between 9-*O* and terminal -CH_3 group, derivatives' stability was increased. Furthermore, they also reported that incorprating diglucose moiety at 9-*O* position of berberine showed excellent anti-diabetic activity than monosaccharide counterparts (Han et al., 2019); (Wang et al., 2020).

Sobova and co-workers have demonstrated the neuroprotective activity of berberine derivatives. Their results revealed that attachment of benzyl ring at 9-*O* position not only enhanced lipophilicity, but also showed better anti-Alzheimer effect. Moreover, the introduction of electron-withdrawing groups at position 4 of the benzyl moiety (such as NO_2 or CF_3) lead to the enhancement of prolyl oligopeptidase inhibition (Sobolova et al., 2020). Ribaudo *et al.* synthesised and studied some semi-synthetic berberine derivatives as multi-target anti-parkinson agents using a set of in silico tools. Their preliminary studies showed that the compounds had good inhibitory activity on MAO-B in vitro. Also, the derivatives showed very strong in silico binder of PDE4, α-synuclein and MAO-B (Ribaudo et al., 2018). Franceschin *et al.* synthesized various synthetic piperidino berberine derivatives and compared them with various G-quadruplex DNA structures and its ability to inhibit telomerase. It was found that piperidino-berberine increases G-quadruplex stability compared to berberine. Furthermore, berberine derivatives were able to stabilize a preformed G-quadruplex structure at 50 mM K^+, but are unable to induce an analogous monomeric G-quadruplex structure with 5 mM K^+ (Franceschin et al., 2006). In continuation of their previous work, recently, they reported selective interactions of synthetic derivatives of two natural compounds, berberine and palmatine, with DNA G-quadruplex structures. It was observed that compounds with dipeptidyl side chains were better stabilizers. Besides, the study clearly showed side chains are essential to improve their interaction with GqS and increase the stability of the complex structures formed because this may represent the base of action to treat several tumors (Franceschin et al., 2018).

8. Applications

8.1. Anti-diabetic effect of berberine

Chen *et al.* first demonstrated the hypoglycemic effects of berberine in alloxan diabetic mice and spontaneously diabetic KK-A^y (a mice strain commonly used as animal model in type 2 diabetes experiments) mice in 1986 (Chen and Xie, 1986). Since then, a plethora of experiment proposed several mechanisms of action, including alleviation in insulin resistance, promotion of glucose absorption and glycolysis, inhibition of gluconeogenesis, promotes insulin secretion, inhibition of various key enzymes (Dou et al., 2021); (Xu et al., 2021). Berberine exerts its anti-diabetic activity by activating AMP-activated protein kinase (AMPK), enhances islet functions, and improves insulin sensitivity. Based on various experimental studies, it has been revealed that activation of AMPK increases insulin activity, enhances the glucose absorption process, oxidation of free fatty acids, and synthesis of GLUT4. As GLP receptors have a crucial function in the survival of β-islet cells, berberine increases GLP-1 and glucose transporter-4 (GLUT-4) levels secretion, thereby accelerating secretion of insulin (Raju et al., 2019). Berberine suppresses the mitochondrial respiratory chain complex I and therefore inhibit ATP synthesis, thereby causing glucose metabolism resulting in glycolysis. It also accelerates the uptake of glucose in muscle cells, adipose tissues by stimulating both GLUT-4 and retinol-binding protein-4. Besides, it also affects phosphorylation of insulin receptor substrate-1, thereby resulting in decreased insulin resistance (Cicero and Tartagni, 2012). According to a clinical study, it has been observed that berberine alleviated fasting as well as postprandial glucose levels in plasma by 1.4 and 3.1 mmol/liter respectively at the end of 3 months; which indicates it has significant glucose-lowering activity (Zhang et al., 2008). It has been reported that various inflammatory factors are involved in the development of insulin resistance, such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), IL-1, and reactive oxygen species (Hirabara et al., 2012). Berberine plays a significant role in reducing the expression of proinflammatory mediators and acute-phase proteins, including IL-6, IL-1β, TNF-α, inducible nitric oxide synthase (iNOS), monocyte chemoattractant protein-1 (MCP-1), cyclooxygenase-2 (COX-2), C-reactive protein (CRP), nuclear factor-κB (NF-κB), chemokine (C-C motif) receptor 2 (CCR2), and matrix metalloprotease 9 (MMP-9) (**Fig. 7**). Berberine has shown anti-inflammatory effect in macrophages by suppressing the phosphorylation of MAPKs, including p38, c-Jun N-terminal kinase (JNK), and extracellular signal-regulated kinase (ERK) which is dependent on AMPK activation (Ma et al., 2018).

8.2. Antioxidant activity of berberine

Production of Reactive oxidative species (ROS) during various oxidative processes can lead to many chronic inflammatory diseases, cause damage to DNA, cells and proteins. There are various oxidative stress markers and antioxidant enzymes such as malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH) and glutathione peroxidase (GSH-Px) .Berberine decreases MDA levels and increases the levels of SOD, GSH and GSH-Px, which aids to scavenge the excessive free radicals and mitigate oxidative stress (Li et al., 2014). It has been found

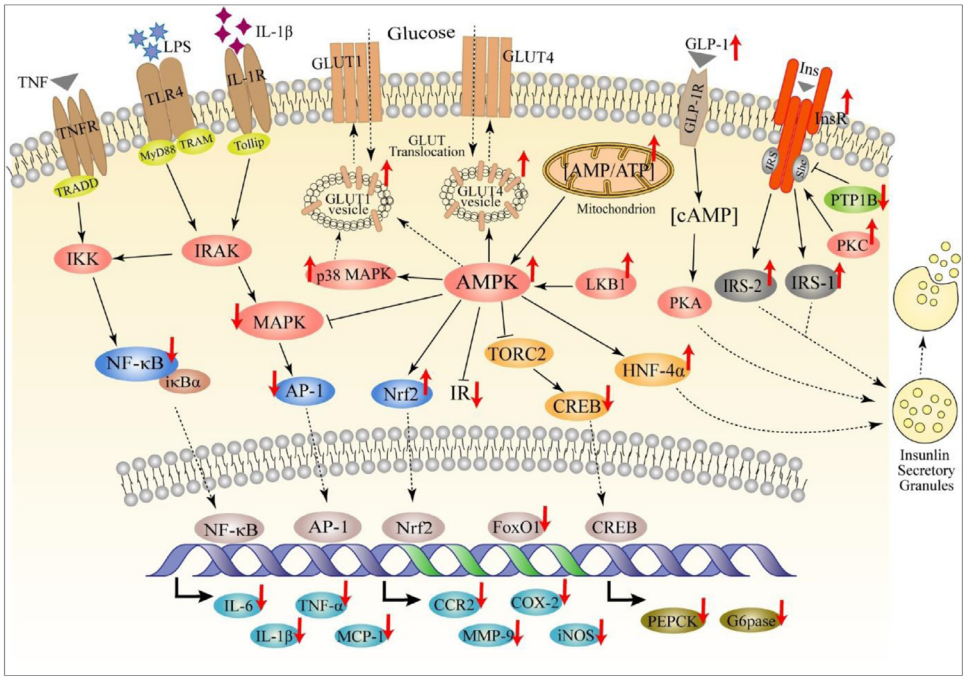

Fig. 7. Targets and Mechanism of berberine's hypoglycemic activity: Berberine increases GLUT1 and GLUT4 translocation and activates the AMPK pathway, causing glycolysis. Expression of key gluconeogenic genes, including PEPCK and G6Pase, was decreased, and hepatic gluconeogenesis was suppressed by berberine. Berberine also decreases the production of inflammatory factors IL-6, IL-1β, TLR4, TNF-α, etc (Xu et al., 2021).

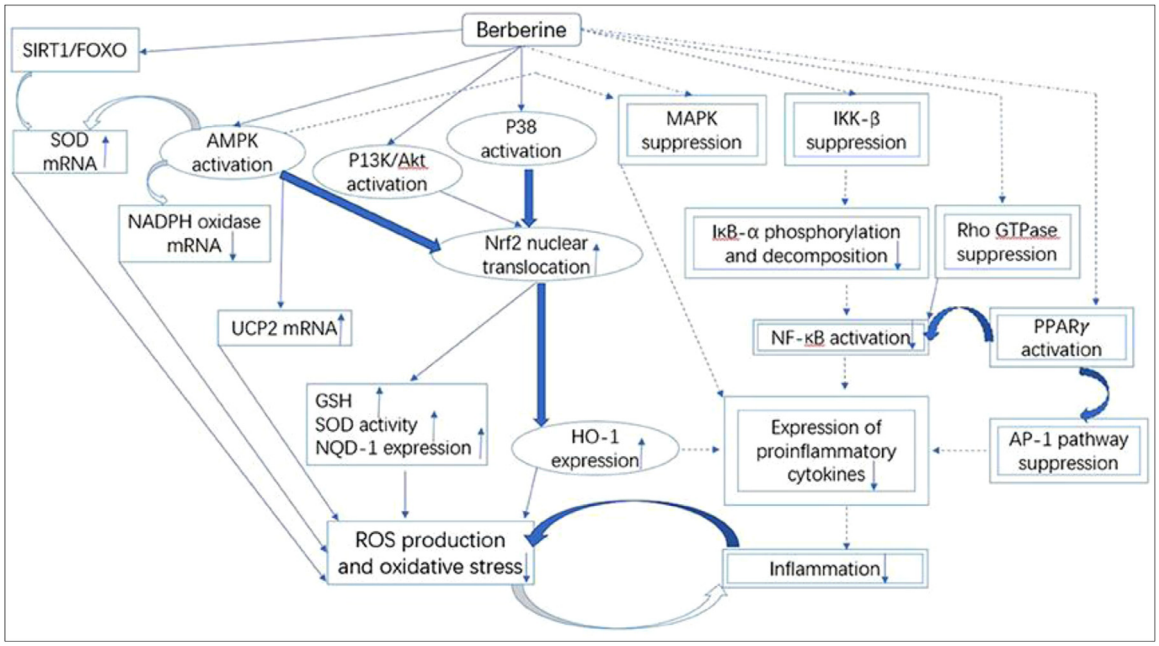

Fig. 8. Antioxidant mechanism of berberine: Berberine inhibits oxidative stress by upregulation of SOD, UCP2, GSH, NQD-1, HO-1 expression and downregulation of NADPH oxidase expression, via SIRT1/FOXO or AMPK pathway (Ma et al., 2018).

that berberine exerts antioxidant activity in a concentration dependent manner by effectively scavenging 2, 2-azino bis (3-ethylbenzothiazoline-6-sulfonate) (ABTS), nitric oxide(NO), 2, 2-diphenyl 1-picrylhydrazyl (DPPH) radicals, as well as suppresses lipid peroxidation (Gan, 2016). Moreover, berberine also acts to alleviate oxidative stress through the Pl3K/Akt/ Bcl-2 and Nrf2/HO-1 (**Fig. 8**). Activation these pathways by berberine induces nuclear translocation of Nrf2 that can activate antioxidant enzyme expression, increase the contents of GSH and SOD in cells and decrease the rate of ROS generation as well as oxidative stress (Li et al., 2014, Rajasekhar et al., 2020). AMPK pathway is also involved and plays a crucial role in the antioxidant activity of berberine. NADPH oxidase is responsible for generating ROS. Thus, the NADPH oxidase enzyme is thus negatively regulated upon activation of the AMPK pathway (Imenshahidi and Hosseinzadeh, 2020). Zhu *et al.*, in their study have reported that berberine increased the expression levels of a deacetylase, Sirtuin 1 (SIRT1), which shows antioxidant activity in oxidative stress process (Zhu et al., 2013).

8.3. *Anti-viral activity of berberine*

Berberine's antiviral activity has been clinically reported against herpes viruses, influenza viruses and respiratory syncytial viruses. One of the mechanisms is that berberine inhibits viral proliferation by modulating NF-κB pathway. It has also been documented that during respiratory

syncytial virus (RSV) infection, BBR significantly reduces p38 MAPK phosphorylation. (Yan et al., 2018). Recently, Shao and co-workers have demonstrated that berberine inhibits entry of HIV-1 effectively and exerts dose-dependent anti-viral activity with IC_{50} values ranging from 5.51 to 10.250μg/ml. (Shao et al., 2020). In another study, the antiviral activity of berberine was observed with an EC_{50} of 0.13μM against HIV-1 NL 4.3 virus in CEM-GFP cell line (a human reporter T-cell line) (Ts et al., 2020). Yan *et al.* in their study evaluated in vivo and in vitro the antiviral activity of berberine against influenza A/FM1/1/47 (H1N1 strain) and reported that berberine significantly reduced necrosis, alleviated pulmonary inflammation, suppressed viral replication and inflammatory infiltration of cells caused by a viral infection in mice (Yan et al., 2018). Various noteworthy and breakthrough research studies have proposed that berberine can be a potential drug candidate to treat COVID-19. For instance, Pizzorno *et al.*, in their study, evaluated the antiviral activity of several single and combined repurposable drugs against SARS-CoV-2, including berberine. They reported berberine's antiviral activity in Vero E6 cells model of SARS-CoV-2 infection with EC_{50} value of 10.6μM (Pizzorno et al., 2020). Another study confirmed their results and added validation to this finding in physiologically relevant primary nasal epithelial cells. Varghese and co-workers recently investigated the in-vitro antiviral activity of berberine and obatoclax (OLX) against Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections in Vero E6 cells as well as in primary human nasal epithelial cells. They reported that both berberine and OLX were effective against SARS-CoV-2 infection in Vero E6 cells at micromolar (EC_{50} value=10.7μM) and sub micromolar (EC_{50} value=0.06μM) concentration respectively. Further investigating on primary human nasal epithelial cells, they concluded that OLX is effective at early steps of the viral life cycle, likely interfering with entry processes, whereas berberine, on the other hand, although does not directly inhibit viral RNA replication per se, but it strongly affects the production of infectious virus as well as the infectivity of newly produced virions and acts on the later stages (Varghese et al., 2021). Pro-inflammatory cytokines like IL-6, IL-1α/β, TNF-α, IL-8, and MCP-1 (CCL2) promote the severity of disease and tissue damage in COVID-19. The major cause of mortality is associated with hyper-inflammation resulting in a cytokine storm. For e.g., Wang *et al.* developed an orally available immunotherapeutic-berberine nanomedicine named NIT-X. They investigated the effect of berberine/NIT-X on the expression of different cytokines and chemokine in infected Calu3 cells. It was found that berberine/NIT-X at 20 μg/mL significantly decreased the expression of pro-inflammatory cytokines and chemokines including IL-1α, IL-8, IL-6 and CCL-2 which reduce the risk of cytokine storm and pneumonia in COVID-19. In addition, their molecular docking analysis revealed that berberine significantly suppressed viral entry host receptor ACE2 and TMPSS2 (Wang et al., 2021). This study suggest that berberine can inhibit various proinflammatory cytokines and protect against tissue damage during viral infection.

8.4. Anti-cancer benefits of berberine

There are a plethora of studies conducted on berberine; therefore, researchers have proposed various mechanisms to elucidate its anti-cancer activity. Berberine stimulates the mitochondrial apoptotic pathway, modifies the action of members of the Bcl-2, activates caspases and induces PARP-1 (poly(ADP-ribose) polymerase-1) cleavage, thus inducing the permeablization of the outer membrane of mitochondria and, subsequently, releasing numerous apoptogenic factors such as cytochrome c or Apoptosis Inducing Factor (AIF). This results in the demolishing of the cells, and also causes chromatin condensation as well as DNA fragmentation (Domingo et al., 2010). Furthermore, it also interferes with the tumor progression and invasion process, presumably by inhibiting main invasion pathway factors such as 12-O-tetradecanoylphorbol 13-acetate (TPA), GTPase, PE2 receptor agonist, epithelial-to-mesenchymal transformation mediated by TGF-β1, and ezrin-mediated by Rho kinase (Ortiz et al., 2014). Berberine shows synergistic activity when used in combination with various conventional drugs and herbs . For example, Ren *et al.*, in their study, have reported cell cycle arrest at the G2/M phase, apoptosis and inhibition of cell growth with increased concentration of intracellular ROS in oesophageal cancer cells when berberine was co-administered with galangin (Ren et al., 2016). In another study, a synergistic anti-proliferation effect was observed when the theophylline and berberine combination was administered to MDA-MB-231breast cancer cells (commonly used cell line to model late-stage breast cancer) as berberine IC_{50} decreased to 50 μM and the cell cycle was arrested at the G2/M level. This induced apoptosis via intrinsic apoptotic pathway (Hashemi- Niasari et al., 2018).

8.5. Anti-obesity effect of Berberine

Berberine is a potential drug for obesity that shows anti-obesity activity in several ways such as by inhibiting adipogenesis, stimulating thermogenesis of adipose tissue and energy consumption, decreasing weight gain, reducing fibrosis of adipose tissue and inflammation (Xu et al., 2021). This antiobesity effect is due to its ability to significantly reduce the size and amount of lipid droplets in the 3T3-L1 (cell line derived from mouse 3T3 cells) adipocyte cell line (Och et al., 2020). By stimulating AMPK activity and fatty acid oxidation, berberine reduces the levels of hepatic and plasma triglycerides, cholesterol (Habtemariam, 2020) Also, a positive effect on the gene regulation process was established for the absorption of cholesterol at a daily dosage of 300 mg in humans has been identified, and an increase in glucose accumulation at a dose of 1.0 g daily has also been observed. (Ilyas et al., 2020). According to a recent study, it has been observed that berberine also improves lipid dysregulation in obesity by regulating the central obesity-related pathway (Park et al., 2020).

8.6. Miscellaneous applications

Berberine shows anti-inflammatory effects by inhibiting inflammatory cell infiltration, the production of TNF-α, IL-1β, and IL-6, and the activation of the TLR4/NF-κB signalling pathway (Li et al., 2019); (Shen et al., 2020); (Wang et al., 2018). Berberine exerts antidepressant activity by influencing BDNF-eEF_2 (Brain derived neurotrophic factor-Eukaryotic elongation factor 2) pathway in the hippocampus, and CREB (Cyclic AMP Response Element-Binding protein) signalling in the frontal cortex; 5-HT_2 receptors activation may partially contribute in antidepressant effects of berberine (Fan et al., 2017, Fan et al., 2019); (Peng et al., 2007). Several studies have reported that berberine exerts neuroprotective effects by inhibiting p-38 MAPK levels (p38 mitogen-activated protein kinase), activating VEFG (vascular endothelial growth factor) pathway, thereby improving learning and memory (Liu et al., 2019); (Wang et al., 2018). Also, it possesses AChE and BChE inhibitory activity, abilities to reduce serum cholesterol and amyloid-β peptide (Aβ) aggregation, which can reduce the risk of Alzheimer's disease (AD) (Imenshahidi and Hosseinzadeh, 2020; Ji and Shen, 2011). Recently Lin *et al.* reported in their study that co-administration of berberine and curcumin showed synergistic activity in improving cognitive functions in AD mouse model and significantly improves the symptoms of AD in mice (Lin et al., 2020). Pierpaoli and co-workers investigate the *in vivo* effects of berberine administration on septic death induced by intraperitoneal *Escherichia coli* injection. They reported that a single 5 mg/kg dose of BBR increases the survival of septic mice. Also, berberine administration improves the antimicrobial efficacy of Imipenem. Besides, berberine pre-treatment prevents improvements of berberine therapy without affecting the pro-survival effects of the Imipenem (Pierpaoli et al., 2020)

9. Toxicological aspects of berberine

Depending upon the organism and the route of administration, the toxicity effects (LD_{50} values) of berberine vary. LD_{50} value of 2,600

Table 5
Summary of recent patents on berberine.

Sr no	Inventors	Patent number	Year	Title	References
1	Peixuan, D.,Hemin,G., *et al.*	AU2020102554A4	2020	A method of treatment of atopic dermatitis on berberine	(Peixuan Duan et al., 2020)
2	Mingfeng.Q., Jing.S.,*et al.*	CN108113977B	2020	Preparation method and application of gelatin-loaded berberine hydrochloride nanoparticles encapsulated by erythrocyte membranes	(Mingfeng et al., 2020)
3	Yongju.X., Yutao T., *et al.*	US10577379B10	2020	Fenofibric acid salt with berberine or its analogues, crystalline forms, methods of preparation, and applications	(Xie et al., 2020)
4	Tan, X.,Ding.Y.,*et al.*	CN110551118B	2019	Method for extracting berberine from phellodendron amurense	(Xinzhu et al., 2019)
5	Houlei.T., Wei.W., *et al.*	US10285969B2	2019	Mangiferin-6-O-berberine salt and preparation method and use thereof	(Houlei Teng et al., 2019)
6	Xiaoyuing.L., Changshun.L., *et al.*	CN104825389B	2018	A kind of Berberine hydrochloride self-micro emulsion formulation and preparation method thereof	(Long Xiaoying et al., 2018)
7	Michael Holstein and Eric Huntingtom	US446100B2	2016	Dietary supplements and formulations	(Holstein and Huntingtom, 2016)

mg/kg in mice was reported upon oral administration of powdered root of *B. vulgaris* . When a fraction of root extract was administered orally in rat and mice, LD_{50} value of 1,280 and 520 mg/kg were obtained respectively. In case of pure berberine, LD_{50} value of 23mg/kg was reported via intraperitoneal route, whereas, upon oral administration, LD_{50} value was found to be 329mg/kg. In rats, LD_{50} value of berberine sulphate is 205mg/kg when administered via intraperitoneal route, whereas, in cats, berberine sulphate at a dose of 50/100mg/kg (orally) resulted in haemorrhagic inflammatory conditions in both small as well as the large intestine. Berberine causes vomiting in cats within 6–8 h upon administering orally at a dose of 100mg/kg; and was proved to be fatal when the same dose was continued for 8-10 days (Singh and Sharma, 2018). Mahmoudi and co-workers investigated immunotoxic effects of berberine in mice, and reported that 5mg/kg influenced the lymphocytes proliferation and also additionally delayed-type hypersensitivity response observed, while 10mg/kg lead to suppression of both humoral and cellular immune functions (Mahmoudi et al., 2016). In another study, LD_{50} of berberine was investigated through three different routes of administration namely intravenous (IV) injection, intraperitoneal (IP) injection, and intragastric (IG) oral administration. Their results have reported the LD_{50} values to be 9.0386 and 57.6103 mg/kg, from IV and IP route respectively; however, surprisingly, no LD_{50} value was established in the IG group because the absorption of berberine is limited by oral route, therefore, explaining the difficulty in obtaining an LD_{50} of berberine for IG injection (Kheir et al., 2010). According to a clinical study, when type 2 diabetes patients were treated with berberine at a dose of 500mg three times/day for 13 weeks, various GI side effects such as constipation, diarrhoea, abdominal pain, flatulence were reported by 34.5% patients. Berberine (50 μM) causes cell death in human keratinocytes (HaCaT) (80% decrease in cell viability) and DNA damage in the form of single-strand breaks, when exposed to UV-A radiations, contributing to phototoxicity and genotoxicity. (Kamrani Rad et al., 2017). A study showed haemolysis of isolated red blood cells in G6PD (glucose-6-phosphate dehydrogenase) deficient patients during in vitro treatment with a Rhizoma coptidis extract (Ho et al., 2014). Berberine can lead to cardiotoxicity, with functional bradycardia. To justify, according to a clinical case, a 53-year-old man admitted to the emergency department for fatigue, dyspnoea upon exertion and bradycardia, six days after starting a berberine-containing product to treat hypercholesterolaemia, was reported. Therefore, this case study suggests that berberine's use should be carefully weighed in hypervagotonic people due to the drug's bradycardic and antiarrhythmic properties, which could became proarrhythmic, exposing patients to potential health risks (Cannillo et al., 2013). Because of its ability to inhibit the hERG (human ether-a-go-go-related gene) potassium channel, a target of numerous antiarrhythmic drugs, berberine can also induce cardiotoxicity (Xu et al., 2017). Thus, to identify safe conditions of use, the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) recently published a report on the safety of use of berberine-containing plants in the composition of food supplements. Due to the poor quality of the available toxicological studies, only an indicative toxicity value (iTV) of 1.7 μg/kg bw/day was proposed. iTV corresponds to a dose of 0.1 mg/day for a 60 kg individual, which is likely exceeded for many food supplements on the market. ANSES reiterates that an iTV is an indicative toxicological benchmark that, while less robust than a toxicity reference value (TRV), can nonetheless be used for temporary risk management purposes, pending the determination of a TRV based on good-quality toxicological studies. The report also suggested a critical dose ($LOAEL_{HED}$) of berberine: 1.25 mg/kg bw/day (ANSES 2019).

10. Patents on berberine

According to the literature, patents on berberine are categorized into three categories- patents containing berberine derivatives, patents containing purified berberine compositions and patents relating to natural extracts containing berberine or berberine containing plants. Furthermore, each category is further divided into four distinct categorize such as patent applications for the treatment of conditions such as inflammation, metabolic and cardiovascular disorders, cancer and several therapeutic areas, patents on enhancing bioavailability (Singh and Mahajan, 2013). A patent application US9427432B2 describes a pharmaceutical formulation comprising berberine for treatment of skin disorders and methods of use. The cream-based formulation contains berberine concentration of about 0.12%w/w, sodium dodecyl sulfate (SDS) as anionic penetration enhancer, and has pH of about 5.5 and 90% of average particle size of the berberine is less than 10 μm. Furthermore, penetration studies were carried out in mini-pig, and it was found that berberine particles released over 24 hours (Wang et al., 2016). Recently a patent application CA2875506C disclosed the use of berberine in the manufacture of a medicament for improving the gut microbiota population. The patent revealed that berberine suppressed bacteria such as *Helicobacter* that is capable of producing highly active endotoxin. In addition, berberine increased bacteria such as Prevotella, Blautia, Butyricimonas that are relatively abundant and are capable of producing short-chain fatty acid (Zhao et al., 2019). A patent application RU2671492C2 has described a topical berberine containing pharmaceutical formulation for treating rosacea or face related skin disorders. It was found that the invention containing purified berberine at a concentration of more than 0.1% was safe and well-tolerated by patients in the treatment of rosacea and related disorders (Shuen-Lu et al., 2018). Some of the recent patents of berberine are summarized in **Table 5**.

11. Conclusion and future perspectives

There has been continuous growth as well as developing interest in naturally derived products in the current scenario. Several natural drugs from medicinal plants that have a history of medicinal applications have been discovered. Berberine is a versatile drug that possesses a plethora of therapeutic benefits which are noteworthy and established. Agglomerating all the discussions done above, this article has gone some way towards enhancing our understanding of berberine's physicochemical, pharmacological, toxicological properties along with its sources, extraction techniques and intellectual aspects.

Generally, berberine is administered via oral route to treat diabetes, hypertension, hyperlipidemia and many other disorders. However, its absorption is limited due to its hydrophilic nature. Since every lock has a key, thus, as discussed in this review, by using various Nano carriers (e.g., liposomes, dendrimers, nanoemulsions, carbon dots, gold and silver nanoparticles, magnetic mesoporous nanoparticles etc.) for encapsulation and incorporating advanced nanotechnology, particle engineering, polymeric materials during formulation, we can overcome the problem of bioavailability of berberine.

Considering the toxicological aspects, future studies should target developing berberine derivatives that can improve efficacy and mitigate the potential toxicity issues discussed in this review. The current review would serve as a comprehensive compilation of various recent research and review reports of berberine which may provide drug designers and medicinal chemist comprehensive information for the development of clinically useful molecules. The industrial scaling-up of berberine has still been ignored, despite the several successful pre-clinical and in vitro trials. Hence, it is strongly recommended that high-quality, well-designed, large-scale, and multicenter clinical trials should be conducted to evaluate the safety, toxicology profile, clinical utility of berberine to develop berberine formulations on a large scale. Overall, berberine, therefore, justifies the transformation of its multiple therapeutic potentials into a techno-commercial and compliant product.

Declaration of interest

The author declares no conflict of interest.

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