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NANO-ENABLED SYSTEMS FOR THE DELIVERY OF BERBERINE

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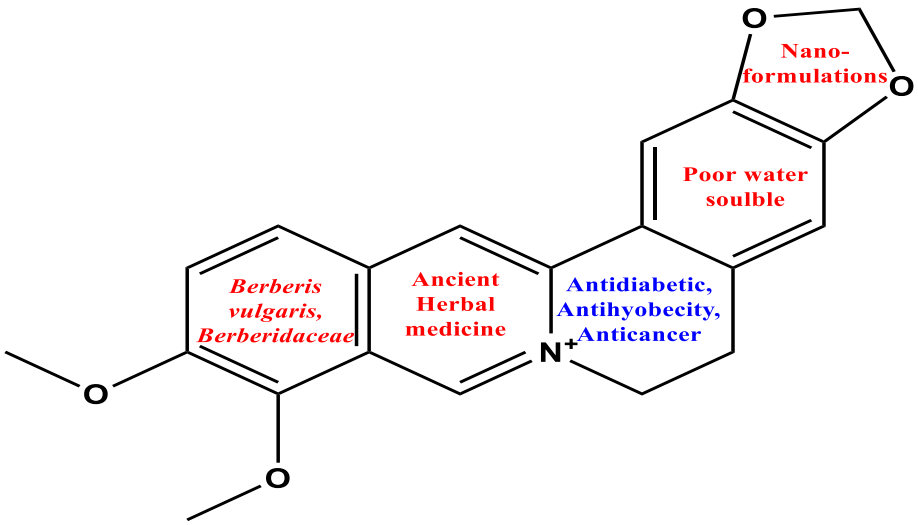
Abstract:
Berberine (BBR) is an alkaloid that belongs to isoquinoline functional group which is further classified as *protoberberines*. From ancient times it has been utilized in hyperlipidaemia & diabetes type II. Various studies performed on Berberine has proved it acts as anticancer by mode of cell necrosis. Due to poor water solubility & minimal bioavailability Berberine fails as effective chemotherapeutic agent. The drug has been combined with other antineoplastic agents to improve activity, but the results were not impressive. Use of adjuvant therapy cannot withstand in today's era, as co-administration may further lead to pharmacological complication in biological system. Nanosizing of Berberine as single bullet or in combination with well-known antineoplastic agent can help to improve distribution & target-based release of chemotherapeutic agents into carcinogenic tissue. In this chapter detailed description, ancient background of Berberine & its application as antineoplastic delineate & importance of nano enabled Berberine is summarized.

Graphical abstract:



Keywords: Berberine; Herbal Medicines; Nanoparticles; Co-delivery Systems; Cancer therapy

1. Introduction

1.1 Berberine

Berberine (BBR) is an alkaloid that belongs to isoquinoline is further classified as *protoberberine*. It is isolated from various plants of ***Berberidaceae*** family such as barberry (*Berberis vulgaris*, Fig. 7.1), Tree turmeric (*Berberis aristate*) which is also called as Chutro, Oregon grape (*Mahonia aguifolium*), along with some plants from ***Ranunculaceae*** family such as Yellow root (*Xanthorhiza simplicissima*), Goldenseal (*Hydrastis canadensis*), Chinese goldthread (*Coptis chinensis*), other than this some trees like Prickly poppy (*Argemone Mexicana, Papaveraceae*), Gulwel (*Tinospora cordifolia, Menispermaceae*), Californian poppy (*Eschscholzia californica*, *Papaveraceae*) (Neag *et. al.* 2018; Wang *et. al.* 2015) Berberine generally isolated from rhizomes, roots and bark of the barberry. It is called by various names in various languages as depicted in Table 7.1 (*flowersindia.com*). Detailed physicochemical properties are depicted in Table 7.2 (*Spinozzi et. al. 2014; Battu et. al. 2010*)

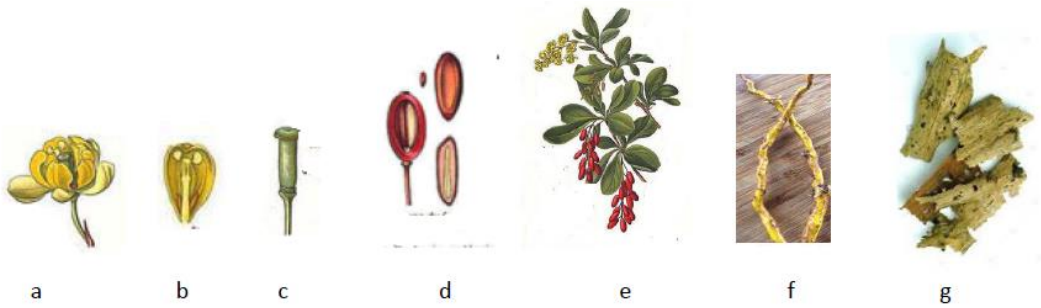


Figure 7.1: Various parts of parts of *Berberis vulgaris, Berberidaceae*

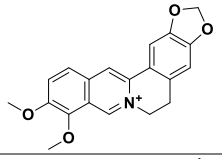
a. Flower bud, b. Flower petal, c. Flower stem d. Seed e. Flowering branch f. Isolated stem g. Recovered stem bark

Table 7.1: Various names of barberry in various languages

Sr. No.	Language	Name
1	English	Indian barberry, tree turmeric
2	Bengal	*Darhaldi*
3	Garhwal	*Kashmoi*
4	Himachal Pradesh	*rasont, kashmal*
5	Hindi	*chitra, dar-hald, rasaut, kashmal*
6	Kerala	*maradarisina, maramanjal*
7	Maharashtra	*Daruhald*
8	Nepal	*chitra, chutro*
9	Punjab	*chitra, kasmal, simlu, sumlu*
10	Tamil Nadu	*mullukala, usikkala*
11	Sanskrit	*daruharidra, darvi, kata, pitadaru, suvarnavarna*

Table 7.2. Physicochemical properties of Berberine

Sr. No.	Parameter	Description
1	Name	Berberine
2	Abbreviation	BBR
3	IUPAC	5,6-Dihydro-9,10-dimethoxybenzo[*g*]-1,3-benzodioxolo[5,6-a] quinolizinium

4	Chemical formula	$C_{20}H_{18}NO_4^+$
5	Structure	
6	Molar Mass	333.36 g mol^{-1}
7	Melting Point	145 °C (293 °F; 418 K)
8	Solubility	Water insoluble, Soluble in Ethanol, DMSO, and Dimethyl formamide (DMF)
9	Appearance	Yellow solid
10	Nature	Amorphous powder
11	Natural source	*Berberis vulgaris, Berberidaceae*
12	Colour index	75160
13	Chemical classification	Isoquinoline alkaloid
14	Medicinal property (s)	Antimicrobial, Herbal supplement, Anticancer (under study)

1.1 Berberine & its metabolites

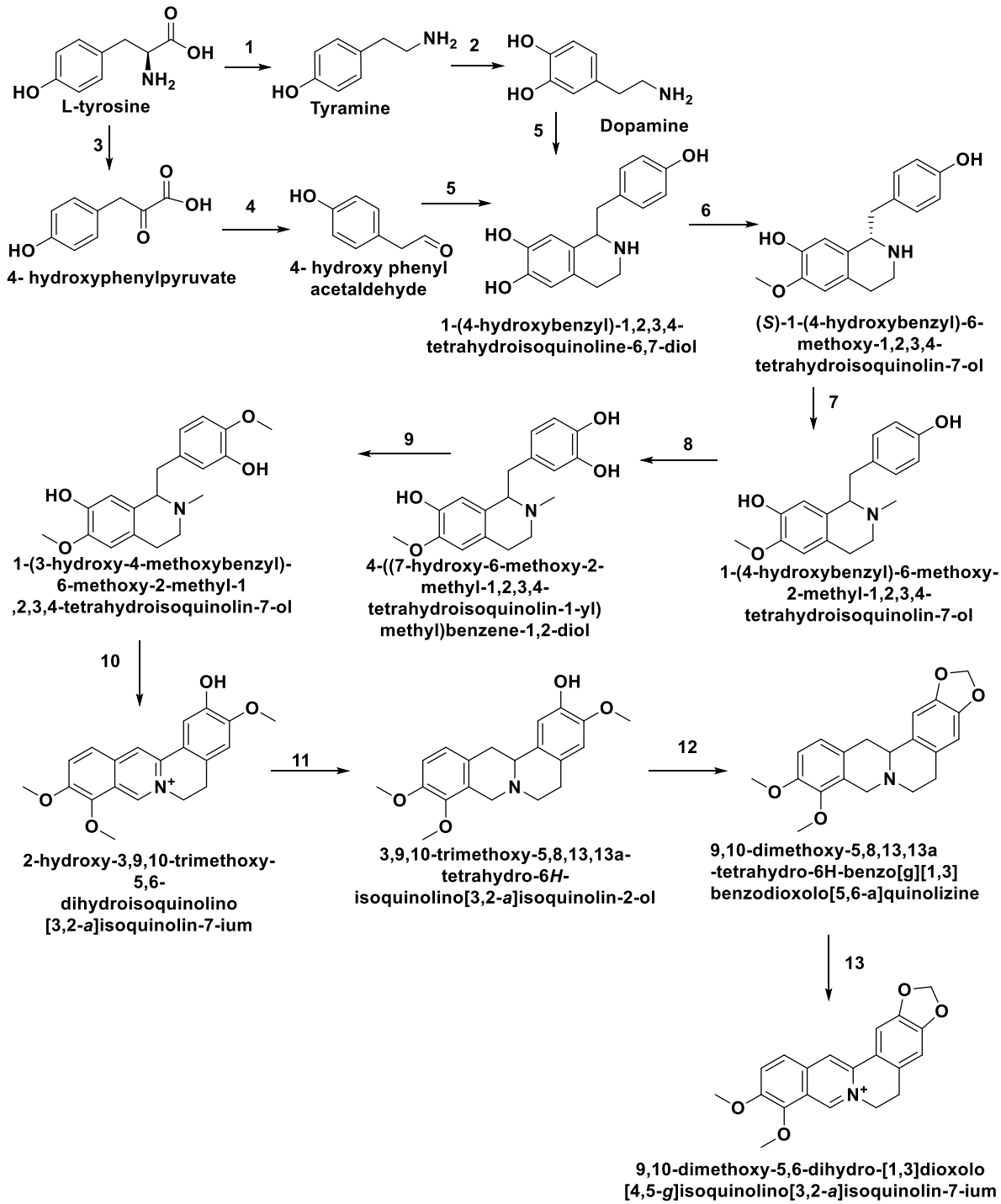
Previously Berberine was administered via oral route. It is extensively metabolised after oral administration, which brings low plasma manifestation. But by observing final pharmacological effects, one can say metabolites of Berberine may also contribute to its activity. Berberine is predominantly metabolised through phase I reactions liker eduction, hydroxylation & demethylation followed by conjugation to various biological molecules like glucose & proteins. Following metabolites are considered active Berberine derivatives *in vivo.*

- De-methyl Berberine
- Berberrubine
- Columbamine

These metabolites exhibit similar biological profiles to Berberine mainly hepatoprotective action. Berberine & its metabolites are active pharmacophores for further biological investigation (*Wang et. al. 2017*).

1.1.2 Biosynthesis of Berberine

Berberine is biosynthesised from L-tyrosine. It is tetracyclic skeleton which contains benzyl tetrahydro isoquinoline. Extra carbon is incorporated in the system. Reticuline is immediate precursor of *protoberberine*. The detailed biosynthetic pathway is as depicted Scheme 1 (*Govindachari, T. R., and K. Nagarajan, 1970 Part I to II; Govindachari, T. R., and K. Nagarajan III to IV*)



Scheme 1. Biosynthesis of Berberine in natural system

1. L-tyrosine decarboxylase, 2. Phenolase, 3. L-tyrosine transaminase, 4. Phydroxy phenyl pyruvate decarboxylase, 5. (S)-nor coclaurine synthase, 6. (S)-adenosyl-L-methionine : nor coclaurine 6OMT, 7. (S)-adenosyl-L-methionine : coclaurine N-methyltransferase, 8. N-methyl coclaurine -49-hydroxylase, 9. S-adenosyl-L-methionine: 39-hydroxy-N-methyl coclaurine 49OMT, 10. Berberine bridge enzyme, 11. S-adenosyl-L methionine : scoulerine 9-O-methyltransferase, 12. (S)-canadine synthase, 13. Tetrahydro protoberberine oxidase

1.2 Historical aspects of Berberine (BBR)

In many traditional medicinal system Berberine has been used as anti-protozoal, chlorotic, cholagogue, cardiotonic, anti-cholinergic, anti-arrhythmic, anti-hypertensive & anti-inflammatory action. It is present in bark, roots & rhizomes of many plants. Plant extracts & decoctions has been

screened for various anti-microbial activities. Specifically, for intestinal parasites. In ancient America Berberine (in form of plant extracts &/or) was used as herbal dietary supplement. In ancient Egyptian system of medicine, it was used it to prevent plagues. (*Kumar et. al. 2008; Gupta et. al. 2009)*
It is called as *daruharidra (***दारुहरिद्रा***)* in hindi. It is native in Himalayan suburbs. The formulation named as rashut (**रशुत**) was used for treatment of ophthalmic disorder, in ulcer as laxative & tonic, which also acts as blood purifier. Other applications of the plant were wound healing, inflammation, skin disease, menohrrhagia, diarrhea, jaundice. (*Mitra et. al. 2011*) It is one of the key elements in vaman (**वमन**) preparation in panchakarma treatment. The effects observed were anti-pyretic, anti-inflammatory, anti-protozoal & hypoglycaemic. (*Wongbutdee et. al. 2009*). ADB Vaidya performed reverse analysis of pharmacological actions of *Berberis aristate* for Berberine, which shows that it binds to DNA further leads to inhibition of DNA cleavage (*Gao et. al. 2020*). In Chinese system of medicine, Berberis is used from last 30 decades. It was prescribed as anti-diabetic agent. In table 7.3 different uses of Berberine in various traditional medicinal systems is depicted.

Table 7.3 Ancient applications of *Berberis aristata*

Sr. No.	Use	System of Medicine(s)
1.	Anti-diabetic	Ayurveda, Chinese
2.	Anti-protozoal	Ayurveda, Chinese, ancient American tribe
3.	Anti-emetic	Ayurveda (Panchkarma)
4.	Laxative	Ayurveda, Chinese
5.	Treatment of plague	Ancient Egyptian system of medicine
6.	Blood purifier	Ayurveda, Chinese
7.	Cardiovascular	Ayurveda, Chinese, ancient American tribe, ancient Egyptian system of medicine
8.	Giardiasis	Ayurveda, Chinese, ancient American tribe, ancient Egyptian system of medicine
9.	Dietary supplement	Ancient American tribe
10.	Diarrhoea	Chinese

Considering knowledge of various traditional medicinal system about Berberine, ethnopharmacological study of *Berberis aristate, Berberidaceae* was performed. As well other numerous plant species containing Berberine were identified and screened for various pharmacological actions (*Hahn et. al. 1975*). In next section detailed pharmacological actions of Berberine has been explained.

1.3 Detailed Pharmacological profile of Berberine.

Till date Berberine has been explored for various pharmacological activities. The common process of study of pharmacological activities of Berberine is isolation from natural sources and then it is standardised by structural analysis further it is optimised for purification & screened *in-vitro, in- vivo & preclinical study on animals* like rats, rabbits & monkeys (*Deepak et. al. 2013*) (figure 7.2.)

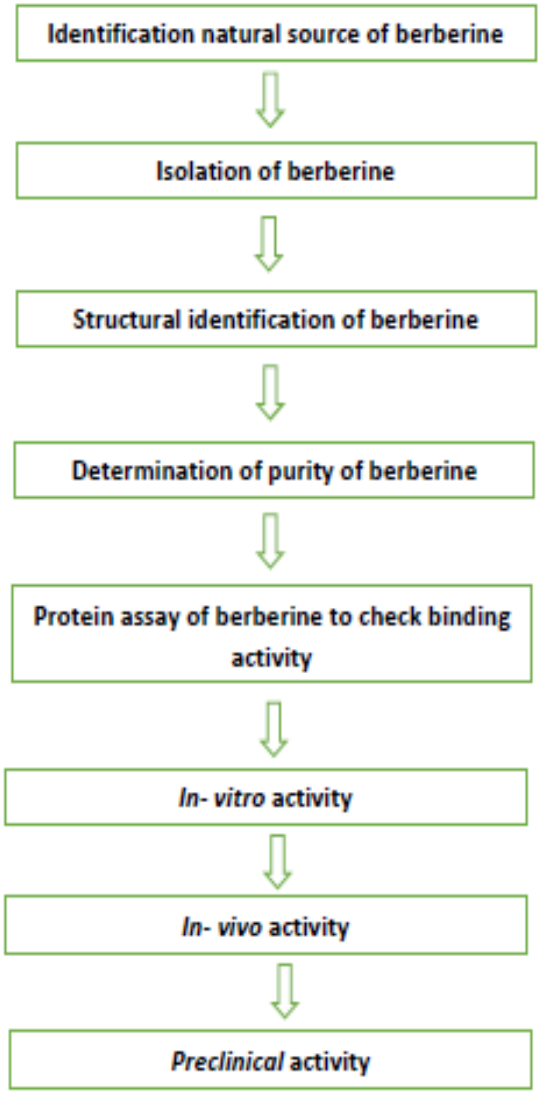


Figure 7.2. Method of pharmacological screening of Berberine

Berberine was first isolated in North America in 1917 from goldenseal. It is then screened for various pharmacological activities namely cardiovascular, neuroprotective, anti-microbial, hepatoprotective & anticancer activity. (*Kim et. al. 2011*)

1.3.1 Cardiovascular activity

Berberine and its other derivatives (tetrahydro-Berberine and 8-oxoBerberine) were screened for various cardiovascular activities. In this study Berberine found to have antiarrhythmic, vasodilator, negative chronotropic & positive ionotropic effect. Further mechanistic study predicted that Berberine blocks K^+ & stimulation of Na^+-Ca^{2+} exchanger. Extension in duration of ventricular action potential has been recorded. Based on this observation Berberine can be recommended as antiarrhythmic &/or in treatment of Congestive heart failure (*Feng et. al. 2019; Lau et. al. 2001*)

1.3.2 Anti-inflammatory & antioxidant activity

Berberine is effectual anti-inflammatory & antioxidant agent. Berberine lowers reducing oxidative stress related with numerous cellular molecular pathways. It can reduce oxidative stress by following 3 mechanisms, (figure 7.3)

A) Inhibition of oxidative stress via accelaration of supra oxide dismutase (SOD) &
B) Induction of actuation of the Nrf2 transcription i.e. activation of AMPK & P38 pathways.
C) Suppression of inflammation by inhibition of MAPK pathways via AMPK dependent manner, inhibition of typical NF-$_k$8 transcription, hindering the Rho GTPase pathway, which is key factor

in NF- $_k$8 management & impairment of the translation of AP-1, feasible to be intervened by PPARγ stimulation (*Lu et. al. 2020; Paul et. al. 2014; Oshima et. al. 2018; Zeng et. al. 2014; Čerňáková et. al. 2002*)

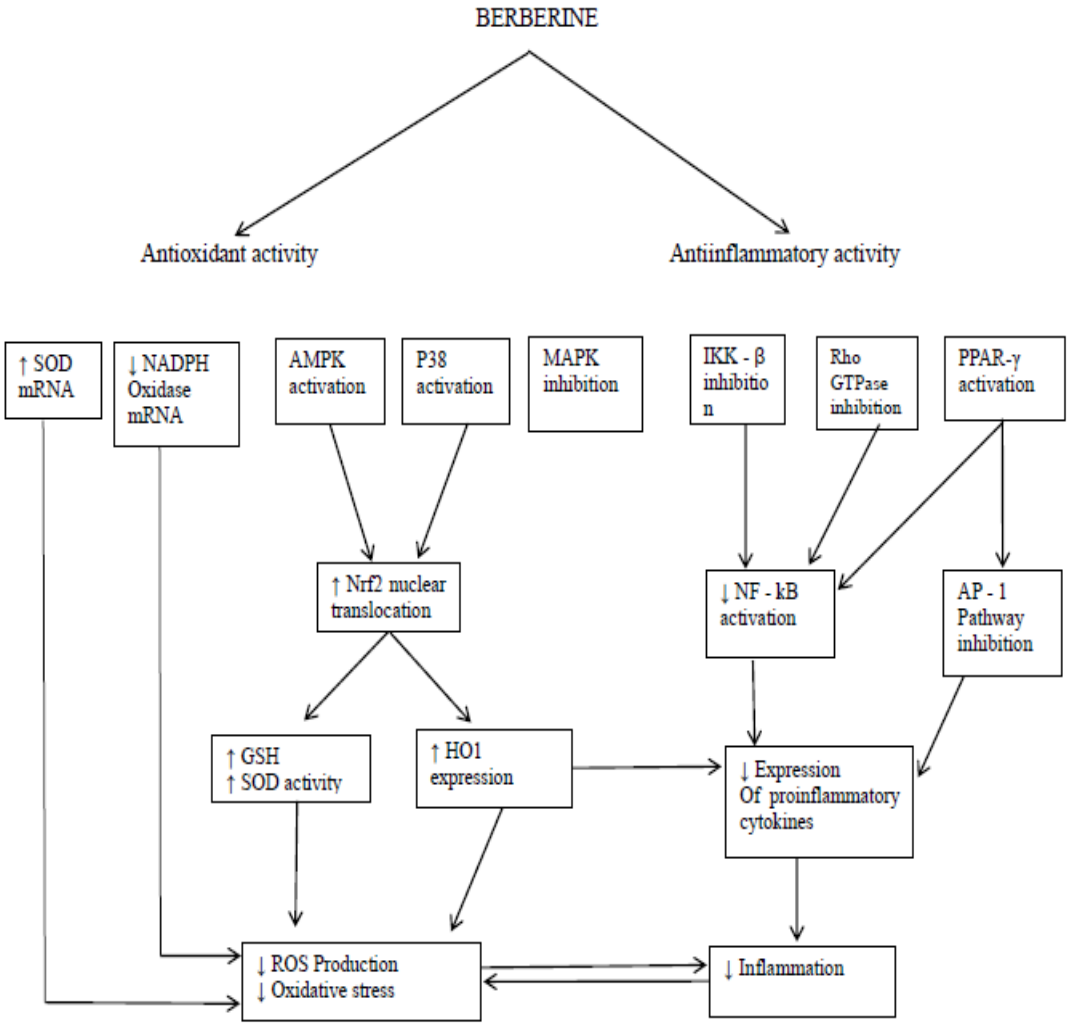


Figure 7.3 Antioxidant & Anti-inflammatory mechanism of Berberine

1.3.3 Effects of Berberine on Glucose Metabolism

There are 2 specified pathways which activates uptake of glucose in peripheral vital tissues. In first pathway insulin is activated by IRS-PI3-kinase & in second activation of AMP activated protein kinase (AMPK), this step precipitates when hypoxia like condition takes place. According to current study Berberine effects are not easy to understand & may lead to activation of both the insulin & the exercise induced glucose uptake. The study also predicted Berberine may inhibit intestinal absorption of glucose, which may contribute to the Berberine's effect on glucose reduction (Fig 7.4) (*Yin et. al. 2002; Leng et. al. 2004; Zang et. al. 2011*).

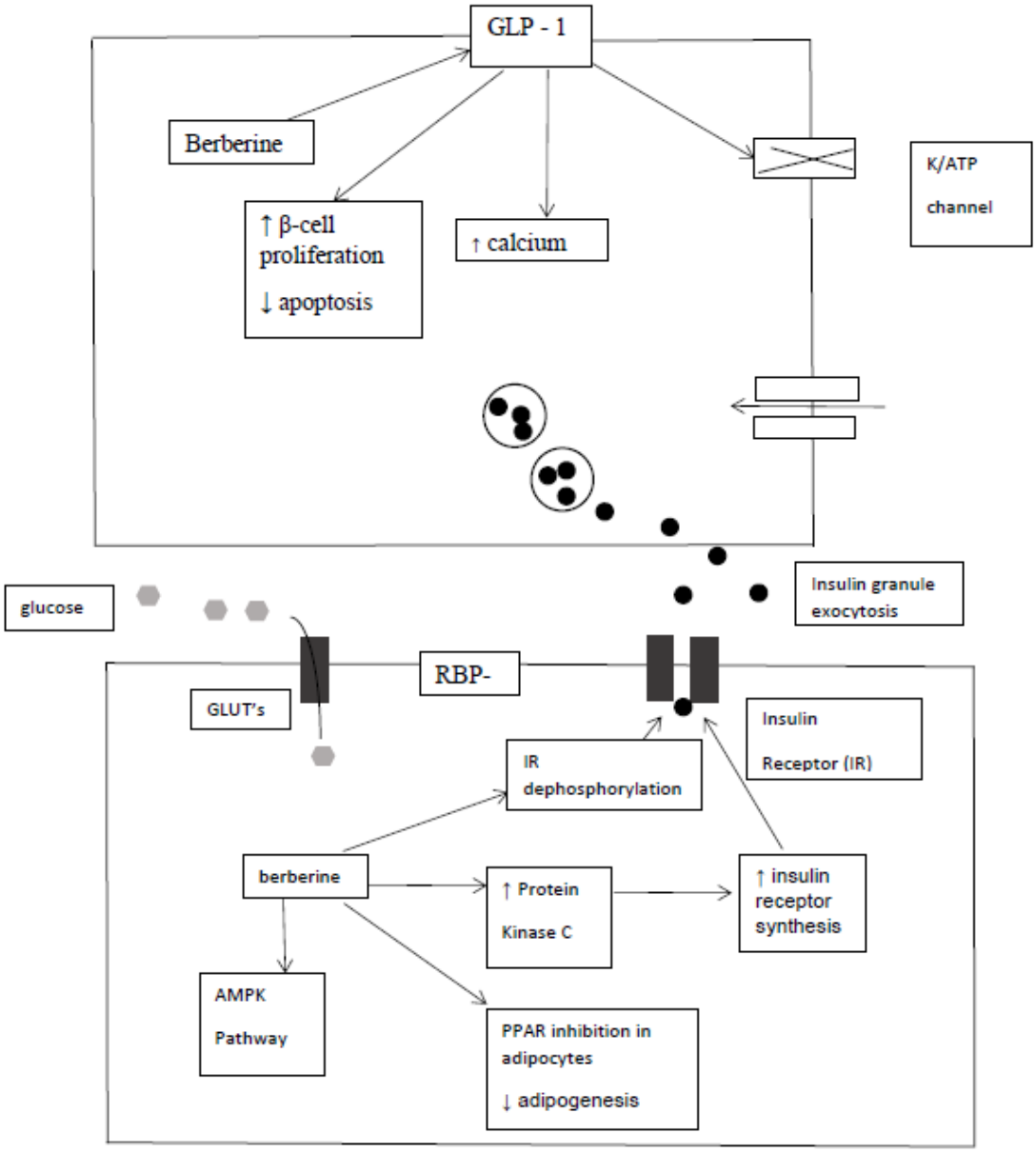


Figure 7.4 Effect of Berberine on Glucose metabolism

1.3.4 Effects of Berberine on Lipid Metabolism

To study effect of Berberine lipid metabolism & vascular health study by systemic study of shuffled clinical trials. The study reported reduction of blood level of cholesterol & triglycerides. The lipid inhibiting action of Berberine can be associated with nutraceutical preparation which will be beneficial in ease of administration. The mechanistic study performed revealed increase in expression of the liver receptor for LDL moderated by hindrance of pro-protein-convertase subtilisinkexin-9 (PCSK9) activity, apart from this upregulation effect through LDL receptor, it is observed that reduction in triglycerides by AMP kinase activation & MAPK/ ERK pathway blocking. (Figure 7. 5) (*Zao et. al. 2017; Sahebhkar et. al. 2017; Jun et. al. 2004*)

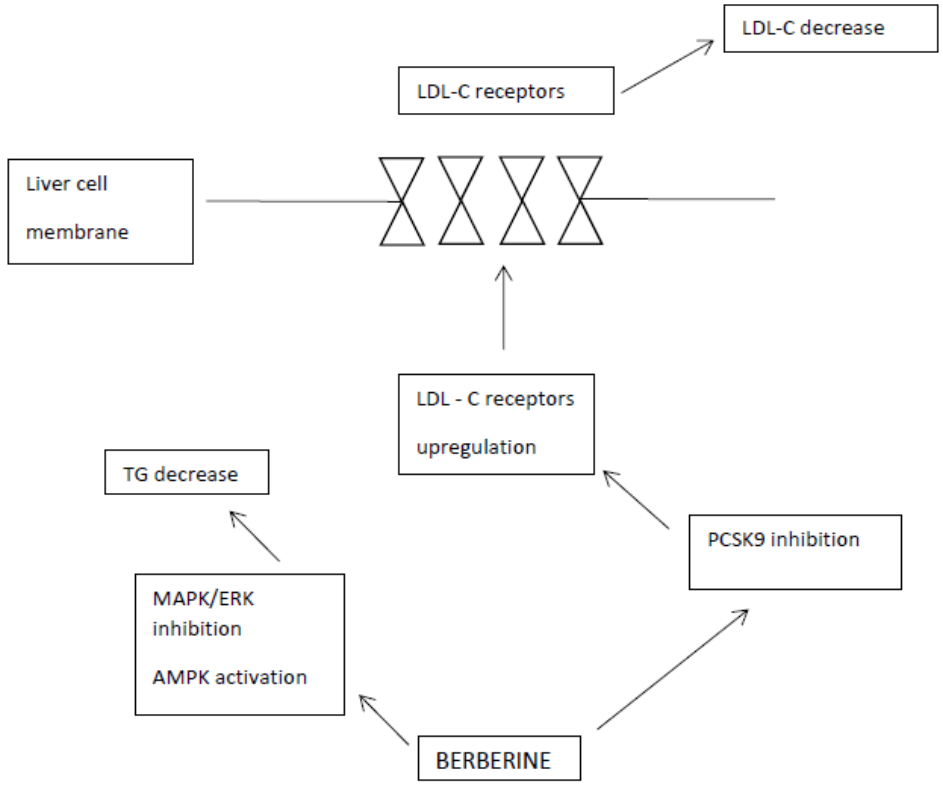


Figure 7.5 Effect of Berberine on lipid metabolism

1.3.5 Effects of Berberine on CNS disorders

Numerous preclinical studies supported feasible role of Berberine in various neuronal disorders like Alzheimer's, Mental depression, Schizophrenia, Cerebral ischemia & Anxiety. In many cases it has been proved in experimental models only. Berberine blocks immobility period in mice in tail-suspension & swim test, which are primarily performed in antidepressant activity for non-dose dependent manner. The mechanism identified exhibits inhibition of MAO-A activity, leads to reduction in degradation of neurotransmitters. It proved that administration of Berberine leads to optimum increase in levels of dopamine, serotonin & norepinephrine which are generally induced by MAO-A enzyme (*Kulkarni et. al. 2010; Fan et. al. 2019; Moghaddam et. al. 2014; Han et. al. 2012*).

1.3.6 Antimicrobial & antiviral activity

Berberine & its naturally occurring analogues obtained from *Mohonia aguifolium* was evaluated against 17 microorganisms. All organisms shown MIC in range of 140-160 µg/ml. it was found moderately active against screened yeast, fungi & bacteria (*Young et. al. 2005; Bandyopadhyay et. al. 2013*).

2. Berberine and Cancer

After potential results of Berberine as antioxidant researchers explored it for anticancer activity. It is observed that Berberine inhibits cell proliferation of different cancer cell lines. Out of various mechanisms reported for Berberine, destruction of carcinogenic cell(s) by induction of apoptosis is best proved. Along with this various research group reported pro-apoptotic effect of Berberine via mitochondria. Alteration of mitochondrial membrane potential inhibition of mitochondrial respiration further precipitation of mitochondrial impairment & regulation Bcl-2 gene expression. Modification in mitochondrial membrane stimulation leads to stimulation of cytochrome c release, which promotes formation of ROS (reactive oxygen species) which trips apoptosis, requires acceleration of caspases & PARP-1 breakdown. Some examples of the pro-apoptotic effect of Berberine are depicted in following table (Table 7.4) (*Guaman et. al. 2014; Ning et. al. 2010*; *Patil et. al. 2010*)

Table 7.4. Mechanistic evaluation of antineoplastic effect of Berberine

Sr. No.	Cell Line & Origin	Effect	Reference
1.	8505C, TPC1 Thyroid carcinoma	Cell cycle arrest	Park *et. al.*
2.	HepG2 Hepatoma	MMP disruption; Cytochrome c release; Bcl-2/Bcl-x_L decrease	Hwang *et. al.*
3.	SiHa, HeLa, Cervical cancer	Caspase activation; Telomerase downregulation	Mahata, *et. al.*
4.	SCC-4, HSC-3 SK-N-SH, SK-N-MC Oral squamous carcinoma Neuroblastoma	Caspase activation; MMP disruption; Cytochrome c release; Cell cycle arrest; ROS production	Lin *et. al.* Ho *et. al.*
5.	HONE-1, NPC, C666-1, Nasopharyngeal carcinoma	Caspase activation; PARP-1 cleavage; STAT3 inhibition; Mcl-1 downregulation	Tsang *et. al.* Kuo *et. al.* Tsang *et. al.*
6.	MCF-7, MDA-MB-231, MDA-MB-468, SK-BR-3 Breast cancer	Caspase activation; PARP-1 cleavage; Cytochrome c release; Cell cycle arrest Caspase activation; PARP-1 cleavage	Patil *et. al.* Kim *et. al.*
7.	IMCE, HCT-116, SW480, SW620, SW613, LNαP, PC-3, DU145 Colorectal cancer	Caspase activation; PARP-1 disruption; ROS production; Cytochrome c release; breakdown of cell cycle; Caspase activation	Ortiz *et. al.*
8.	A431 Epidermoid carcinoma Lymphoma	MMP disruption; Bcl-2/Bcl-x_L decrease	Mantena *et. al.*
9.	U937, HL-6- Leucemia	Caspase activation; ROS production	Letasiova, *et. al.*
10.	A375, Hs29 Melanoma	COX-2 downregulation	Singh, *et. al.*
11.	Panc-1 Pancreatic cancer	TRAIL activation	Refaat, *et. al.*

Based on results collected from numerous studies performed by various researchers, we can conclude that Berberine inhibits cell proliferation and it can be predicted that it could regulate miRNA (microRNA), short non-coding RNA which consist of 20-25 nucleotides sequence produced in nucleus & biological process involved like cell proliferation, development & death. Mis-regulation of miRNA was observed in many human cancer types, they can act either by suppression of tumour or oncogene. In hepatocellular carcinoma, miRNA expression was observed drastically increased which results in conclusion that miRNA is key target for Berberine. This process contributes to reduce cancer cell escalation & induction of cell necrosis. The mechanistic study of miR-21-3p revealed expression of MAT (methionine adenosyl transferase) gene (Fig 7.6).

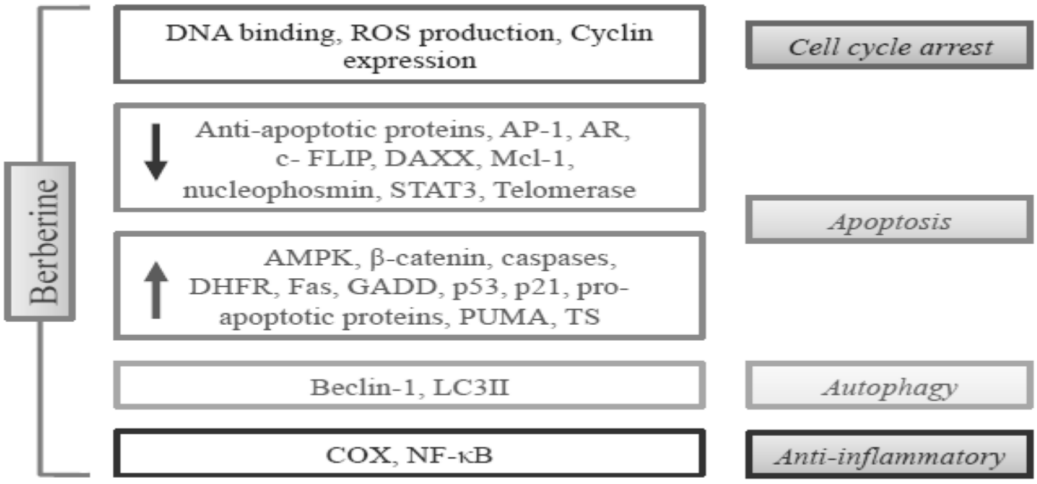


Figure 7.6 Summary of anticancer effect of Berberine

3. Nano-enabled Berberine as anticancer agent

As we had seen various effects of Berberine in last section especially as anticancer agent we can strongly comment that Berberine should be considered for further clinical examination as anticancer drug candidate. But along with promising anticancer activity Berberine also have difficulty in administration due to various physiological properties of it. One of the major drawbacks of Berberine is poor water solubility. Poor bioavailability is also tricky key area in development of formulation of Berberine. (*Hossein et. al. 2020; de Souza et. al. 2020; Kumar et. al. 2020)*

Solubility can be improved by:

- Particle size reduction
- Solid dispersion
- Nano-formulation

With above methods solubility is improved in all cases. But nano-formulation also improves bioavailability. Researchers all over the world explored nano-formulations of Berberine to improve its bioactivity via various formulation methods such as

- Polymeric nanoparticles
  - Poly amido amine (PAMAM) dendrimers
  - Chitosan nanoparticles
  - Dextran nanoparticles
- Metal nanoparticles
- Lipid nanoparticles
- Liposomes
- Carbon based nanomaterials (CBNs')
- Graphene nanoparticles

(*Liu et. al. 2019; Zao et. al. 2012; Ren et. al. 2012; Wang et. al. 2016; Ma et. al. 2013; Choi et. al. 2014; Xin et. al. 2017; Massod et. al. 2016; Bregoli et. al. 2016; Jacob et. al. 2018; Ho et. al. 2017; Batra et. al. 2019*)

3.1 Polymeric nanoparticles

Polymeric nanoparticles are divided into 2 types of biodegradable & non-biodegradable polymers. Preparation of nanoparticles can be customized based on molecular weight, size & hydrophobicity. Behaviour of polymeric nanoparticles is based on composition of Berberine & morphology of formulation. Surface of the formulation used to attach Berberine &/or targeting molecule. Surface functionalisation can be carried out by attaching Berberine/ targeting element to the polymer by covalent bonding. Commonly used polymers are as follows:

- PLGA (polylactic-co glycolic acid)
- PEG (polyethylene glycol)
- PEI (polyethyleneimine)
- PLA (poly lactic acid)
- Chitosan
- Gelatine
- Albumin
- Alginate
- Collagen

(*Díaz et. al. 2013; Li et. al. 2016; Buse et. al. 2010; Kadajji et. al. 2011*)

Bhatnagar *et. al.* (2018) formulated Berberine chloride inclusion complex in PLGA further conjugated with hyaluronic acid. The target was CD44- positive cancer cell. Comparative study was performed with Berberine containing PLGA nanoparticles with free Berberine. Results showed that Berberine nanoparticles with hyaluronic acid showed excellent cytostatic effect to MCF-7 & HeLa cell lines. Further *in-vivo* study into mice infected with Ehrlich ascites carcinoma (EAC) tumor led to accelerated apoptosis, improved viability time, elevated ROS levels (Fig 7.7)

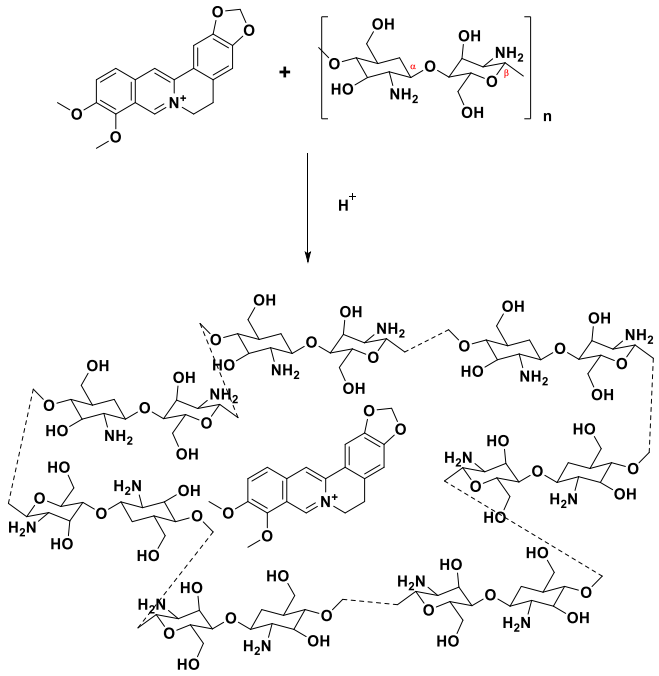
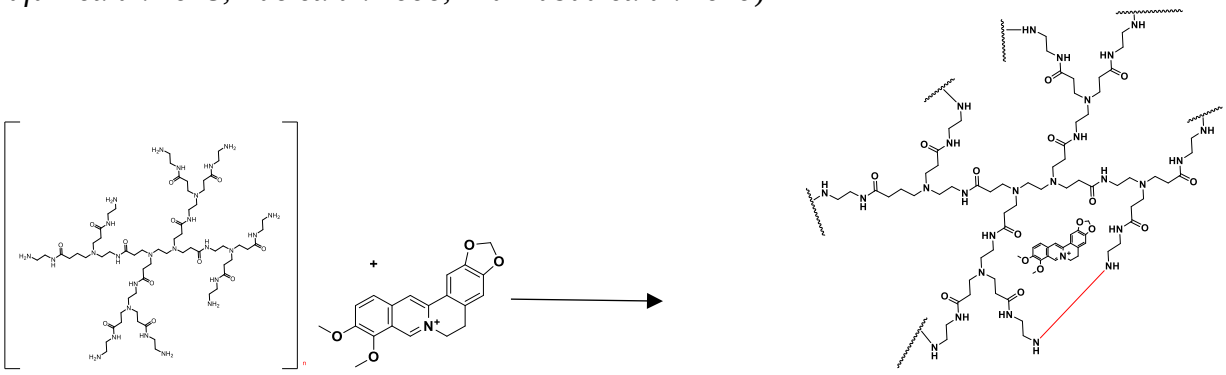


Figure 7.7: General Mechanism of synthesis of Chitosan based nanoparticles.

3.1.1 Berberine containing Poly(amidoamine) PAMAM dendrimers

These dendrimers are highly compatible polymers due to monodispersed, modifiable surface group, well defined architecture & uniformity which make them first choice for formulation in biomedical research. PAMAM nanoparticles have poly substituted structure with numerous functionalities on the exterior of molecule containing Diaminoethane group located interiorly. The coherent size and molecular weight based on the generation number, which states for their consistency (*Ferruti et. al. 2013*).

Gupta *et. al.* synthesized dendrimers of Berberine with PAMAM, in which Berberine was incorporated inside PAMAM (Fig. 7.8). Anticancer activity was investigated against MDA-MB-468 & MCF-7 human breast cancer cells. Comparative study was performed with free Berberine, result showed that PAMAM encapsulated Berberine exhibits higher anticancer effect. (*Wang et. al. 2010; Ghafari et. al. 2018; Zao et. al. 2006; khairiabad et. al. 2020*)



Second generation

PAMAM dendrimer Berberine PAMAM Nanoparticles

Figure 7.8 Mechanism of synthesis of PAMAM nanoparticles

3.1.2 Berberine loaded Chitosan Nanoparticles.

Chitosan is natural polymer isolated from carbohydrates and can be modified in laboratory easily in laboratory to form nanoparticles. As it is natural polymer it is easily degradable in biological system & it is non-toxic. Along with this it is cost effective ingredient. (*Ren et. al. 2016*) It is non-immunogenic & consist of inherent antimicrobial properties, which makes it different from other polymers. Wang *et. al. (2018)* synthesized Berberine loaded nanoparticles of chitosan which further

attached with folic acid which was targeting ligand. The formulation was effective against CNE-1 cells. The formulation inhabits growth & migration of cancerous cells.

3.1.3 Berberine loaded Dextran Nanoparticles.

Iwona *et. al.* (*2019*) synthesised inclusion complex of Berberine with γ-cyclodextrin (GCD) conjugated propylene diamine (PDA). The formulation (GCD-PDA-BBR) was mucoadhesive & resistant to digestion with α-amylase, which leads to form coherent oral formulation. The anticancer activity screened against murine melanoma (B16-F10) cells. It was observed complexed Berberine penetrates lipid membrane easier than actual Berberine. Anticancer activity was improved by 25% in GCD-PDA-BBR formulation.

3.2 Metal Nanoparticles

Metal & metal oxide-based nano-formulations are preferred due to:

- Can be prepared in wide range of size & shapes.
- Accelerated oxidation in aerobic conditions makes compatible to biological system.
- Can be synthesized using silver, gold, zinc & iron oxide.
- Metallic nanoparticles have larger surface area, modifiable to lipophobic with static electricity on the circumference, enabling maximum drug encapsulation (*Fedlheim et. al. 2001; Rao et. al. 2000*).

Pandey *et. al. (2013)* synthesized gold nanoparticles conjugated with folic acid. The formulation was screened against HeLa cancer cells. Comparative study was performed against Vero cells. It was observed that nano-formulation exhibited selective cytotoxicity towards HeLa cell lines, where Berberine exhibited cytotoxic effect on Vero cell lines also. The low toxicity towards Vero cell lines by nano-formulation indicates its selectivity due to attachment of folate to nano-formulation.

Bhanumathi *et. al. (2018)* synthesized biocompatible folic acid- PEG Berberine silver nanoparticles. The silver particles were attached on the surface by using citrate. The formulation was evaluated against MDA-MB-231 breast cancer cells for cytotoxic effect. Induction of apoptosis through generation of ROS, altered gene expression of PI3K, ERK, Bcl-2, Bax along with condensation of nuclei. *In-vivo* antitumor activity performed on mice, showed that significant reduction in tumour growth.

Kim *et. al. (2018)* formulated hybrid nanoparticles based on organic zin-oxide to deliver Berberine in lung cancer. The study was performed on mice. Blood test performed shows negative results for hemotoxicity & hepatotoxicity of formulation after intravenous administration of nanoparticles. The same formulation was screened against A549 human lung adenocarcinoma which showed apoptosis further leads to cell cycle arrest.

3.2.1 Iron-oxide Nanoparticles of Berberine

Ferrous oxide (Fe_2O_3) is an inorganic compound with plasmonic & paramagnetic features has been enormously utilised as medicinal carrier, like selected drug, cancer diagnostics & gene delivery (*Tietze et. al. 2015*). Sreeja *et. al. ()* synthesized Berberine loaded iron oxide nanoparticles & hypoxic sensitizing drug sanazole. Study was performed on solid tumor bearing mice. After oral administration of the synthesized formulation combined effect of Berberine & sanazole produced angiogenesis & tumour regression

3.2.2. Mesoporous Silica Nanoparticles of Berberine

Interior structure of nanoparticles synthesized from mesoporous silica manageable shape & size, which permit desired drug easily enclosed in it, which prevents degradation & undesired drug release. Pores within MSNs can be modified to integrate lipophilic or lipophobic drug candidates to highest cellular internalization (*Sharma et. al. 2018*). Yue *et. al. (2015)* formulated mesoporous *organo-silica* nanoparticles consisting of disulphide bridges as drug transfer system to release Berberine inside HepG2 human liver cancer cells. The formulation showed high drug holding ability, uniform

morphology & biocompatible formulation. When the formulation further enriched with glutathione, it showed improvement in biodegradability & efficient release of Berberine.

3.3 Lipid nanoparticles of Berberine

Lipo-derived nano-formulations possess distinct properties such as

- Controlled drug release,
- Large surface to mass ratio
- Easily encapsulation of sparingly aqueous soluble drugs.

Nano-structured lipid carries, micelles, solid lipid nanoparticles & liposomes are various types of lipid formulation. As solid lipid nanoparticles (SLN) & nanostructured lipid carriers (NLC) can be formulated from natural components via uncomplicated synthetic procedure, commonly chosen for large scale preparation. NLC & SLN are advantageous in cancer therapy. There advantages are as follows:

- Controlled & extended release.
- Compatible with maximum class of drugs.
- Lengthened blood stability.
- Biocompatible easily degrades in biological system.

SLN were developed in initial stages of lipid nano-formulation era, size ranges from nano-meter sub-micron scale (*Riaz et. al. 2018*), NLC synthesized in later era of lipid nano-formulations, which contains unstructured liquid lipids & structured solid lipids, with ability to carry with tremendously lipophilic drugs & release efficiency as compared to SLN (*Dolatabadi et. al 2016*).

Yu *et. al. (2018)* formulated polymeric self-assembled hybrid lipid nanoparticles loaded with Berberine conjugated phospholipid isolated from soyabean to improve oral bioavailability of Berberine. Enhanced lipid solubility was found in the formulation. The formulation was highly stable in biological system. Also, the cytotoxic effect was improved by 30% than normal Berberine.

3.3.1. Liposomes of Berberine

Liposomes are distinctive structural features. They contain bulbous shaped nanovesicles encompassed with bilayers of lipid. They able to enclose both lipophilic & lipophobic drug candidate at a time. They possess isomeric structure to the cell membrane, which helps to deliver content into carcinogenic cell. Nanoliposomes enhances drug stability by limiting interactions occurred with nonrelated biological component, which is also helpful in reduction of adverse actions (*Khan et. al. 2015*). Formation of liposomes makes release of medicine more advantageous on following points.

- Decrease in toxicity.
- Improved inclusion capacity
- Biodegradability
- Eligibility of surface modification, helpful in improvement of circulation halftime in biological systems.

In today's date, many healthcare bodies approved clinical use of nano-liposomal drug delivery. Lin *et. al. (2013)* produced complex of Berberine in liposomes to study anticancer effect of liposome enabled Berberine on HepG2 cells in a murine xenograft model. Drug inclusion capacity of Berberine was improved along with improvement in stability of liposomal formulation by merging DSPC & PEG in liposomal formulation namely. Cytotoxicity of Berberine specifically towards HepG2 cells was higher by 15.7 % by pure Berberine.

Apart from this results, lipo-enabled Berberine activates signalling pathway dependent on caspases-mitochondria, further reduce membrane potential of mitochondria & release of cytochrome C generation of apoptosis. In *in-vivo* experiments Berberine was detected after 72 hrs after injection of formulation, while free Berberine detected in 3 hrs, it proves that lipo- enabled Berberine lengthens time of circulation Berberine in plasma. No adverse reaction was observed in nude mice model.

3.4. Carbon nanoparticles of Berberine

Nanomaterials derived from carbon includes.

- Nanodots of carbon
- Carbon based nanomaterials (CBNs)
- Fullerenes
- Nano tubules of carbon
- Graphene mono structures

Carbon based nanomaterials possess variety of unique optical, thermal, electronic & mechanical properties (*Singh et. al. 2009*).

Peripheral modification of CBNs helps their own dispersion humid surrounding & facilitation of interaction with biotic constituents, hence they can be potentially used for biomedical applications. CBN based Berberine formulation has reported with minimal enhancement against various cancer cell lines. Further study is needed in this type of formulation (*Ali et. al. 2013*).

3.4.1 Carbon Dots (CD's) of Berberine

CD's are advantageous over other formulations because:

- Easy surface modification
- Tuneable optical properties
- High biocompatibility
- Excellent chemical stability

These properties are ideal for treatment of cancer. Their optical properties enable its use as fluorescent imaging & photothermal therapy in many diseases especially in cancer (*Lim et. al 2014*). CD can be complexed ionically & conjugated non-ionically with established antineoplastics & radio diagnostic agents.

Zhang et al. (*2018*) synthesized innovative versatile composite of Berberine-carbon dots which may be incarnated into carcinogenic tissue for directed *in vivo* radio diagnosis. Synthesized nano-formulation able to hinder growth of tumour, with no toxicity to city to noncancerous tissue. Results of screening against MTT cell lines, carbon dots of Berberine reduces sustainability of human lung, breast & liver cancer cells. Carbon dots of Berberine were more toxic to carcinogenic cells than noncarcinogenic tissue mass, illustrating a high level of sensitivity towards carcinogenic tissue. *In-vivo* activity of Berberine carbon dots was more potent than free Berberine.

3.4.2. Graphene nanoparticles of Berberine

Graphene is another form of carbon having 2 D layer of trigonal carbons. The molecular system has numerous advantages in drug release kinetics such as

- Thermal conductivity
- Facile Functionalisation
- Greater surface area
- Mechanical strength

Which makes graphene eligible for solubilization & binding of drug molecules. Maximum dose carrying capacity because of lipophilic or electrodynamic exchange between drug & graphene which makes the drug circulation & delivery modification easily accessible (*Bamrungsap et. al. 2012*). Graphite enabled Berberine show minimal cytotoxicity in comparison with remaining forms CBNs, though its cytotoxicity based on the quantity so that smallest dose of graphene has not exhibited any cytotoxicity (Bhattacharya et. al. 2016).

Thakur *et. al. (2016)* synthesized quantum dots of graphene with cow's milk for delivery of antineoplastic agent & radio imaging probes at a time. They loaded Berberine in the formulation with

cysteamine HCl. The system was efficient to deliver 88% of drug. The formulation was cytotoxic to MDA-MB-231 & HeLa cell lines. It does not exhibited toxicity against L929 tissue.
The results analysed by flow cytometry which showed as, formulation induces apoptosis & G1 phase cell cycle arrest. Author concluded graphene enabled formulations are suitable for delivery antineoplastic agent & it also applicable as illuminating nanoprobes.

4. Nano enabled Berberine in co-delivery of therapeutic agents

Nano formulations can deliver more than 2 remedial agents like:

- Drug
- Genes
- Imaging probes

in various permutation & combination.
Co-delivery systems provides ideal dose of synergistic & additive drug-gene or drug-drug formulation more efficient & intense in comparison to single drug enabled system. As well pharmacological, physical, chemical properties of drug candidates may be contrast & should be considered in translational research i.e., preclinical & clinical studies.
For consideration, drug-drug & drug polymer interaction may lead to uncertain variations in batch synthesis in

- Nano vehicle stability
- Drug loading rates

Dedicated statistical analysis need to perform in determination of overall activity of adjuvant therapy i.e., synergistic or antagonistic effects. Whereas codelivery can modulate biological response due to transition through physiological barriers in biological system, improved medicament percolation in carcinogenic tissue, boosted targeting & bypassing of toxic effect (*Liu et. al. 2019*). When adjuvant drug therapy is applied directly, it may show pharmaceutical incompatibility, unregulated ADMET parameter along with undesirable therapeutic effects leads to failure of treatment. Which results are altered on nanosizing of adjuvant treatment. Altered effects are found as follows:

- Alteration in blood plasma level
- Alteration of physicochemical properties
- Alteration of release kinetics

depending on the nano carrier design. (*Ren et. al. 2016*)
Specified targets are identified interior of cell organelles like

- Cytoplasm
- Nucleus
- Mitochondria

Hence so-delivery systems can release their drugs accurately to these spaces. Lipo-enabled carrier are best suitable carrier for co-delivery (*Shrinivasan et. al. 2016; George et. al. 2019*).
Nanoliposomes, in which lipophobic drugs are possible to enclosed inside watery core, on the other hand extremely lipophilic drugs are possible to encapsulated between lipid bilayer (*Diaz et. al. 2013*).
Tuo et. al. (2016) synthesized Berberine derivative with substitution of C16-alkyl chain at C-9 position. Which were incorporated between bilayer membrane of liposomes through C16 alkyl chain of Berberine, which further conjugated to doxorubicin-folic acid hybrid along with PEG on periphery of liposome. This system provides nano system targeting to mitochondria. Further anticancer screening against doxorubicin resistant MCF-7 cancer tissue in *in vitro* as well as *in vivo*. The formulation exhibited potent cytotoxicity along with accelerated apoptosis in comparison with individual Berberine & doxorubicin.
Formulation increase uptake of Berberine by 15 times, which was drastically more than free doxorubicin. This cumulatively resulted in improved targeting capacity. Also, the nano-formulation improves drug distribution level within tumour tissue, results in suppression of tumour growth in

MCf-7/adr cells grafted in mouse. Finally, we can conclude that constructed nano-system does not target actively or passively, also targets pathways through mitochondria to deliver 9-C16 Berberine & doxorubicin.

Ma *et al.* (*2018*) structured liposome enabled Berberine, & paclitaxel co-loaded Berberine liposomes. The formulations screened against stem cells of breast cancer (CSC) for resistance mechanisms. These cells are responsible for degeneration of breast cancer after treatment. the study was performed on CSC grafted mice & human breast cancer cells. The results exhibited following pathway of mode of action.

- Entry of lipo-enabled Berberine inside CSC
- Blocking of drug efflux transporter
- Specific accumulation in mitochondrial interstitial fluid.
- Retardation of mitochondrial proteins
- Upregulation of Bax
- Downregulation of Bcl-2
- opening of mitochondrial transition aperture
- Cytochrome c release
- Trigger of signalling pathway of mitochondrial apoptosis.

Berberine co-enabled paclitaxel also exhibited same pathway along with improved efficiency in last step compared to individual Berberine & lipo-enabled Berberine. These formulations exhibited maximum antineoplastic *in vivo* effect with complete invasion of tumour.

Zhang *et. al.* (*2019*) synthesized hyaluronic acid conjugated mesoporous magnetic silica nanoparticle of Janus class. The formulation was screened against healthy liver tissue, & HCCs overregulated with CD44- receptor. Improvement antineoplastic of doxorubicin in nano enabled formulation was observed. The mechanistic pathway identified was blocking of Caspase-3-iPLA-COX-2.

The formulation was screened for "phoenix rising" signalling pathway on animal model injected with cancer tissue. The evaluated results concluded Berberine co-enabled doxorubicin can outclass repopulation of carcinogenic tissue, leading to limit regeneration of tumour.

5. Conclusion:

In today's era of modern medicines, target based drug delivery is important due to

- Reduction in dose
- Improved biological activity.
- Overcome side effects.
- Overcome resistance mechanisms by mutation.

From ancient times natural drugs are most important in medicinal chemistry. From the day cancer has been identified, researchers from all fields related to medicines are exploring all possible ways to diagnose & cure cancer.

Chemotherapy is primary method of treatment in all tumour types, it commonly exhibits adverse pharmacological actions, limiting efficacy leading to discontinuation of therapy. The drawback of this therapy led demanding involvement in ancient medicine. Berberine a natural compound belonging to isoquinoline class is predominantly found in stems, roots, bark of barberry. Based on the quantity of Berberine & classification of carcinoma advantageous effects like induction of apoptosis, antiproliferation & arrest of cell cycle has been seen, but due to bad aqueous solubility, vulnerable stability in biological system & impecunious bioavailability of Berberine lead to limitation of its use. Nanotechnology might be helpful in solving this issue. Co delivery of anticancer agents provides a strategy to encounter the hurdles in front of existing therapy like:

- Resistance to drug due to over expression of proteins
- Multi drug resistant tumour

Nano-enabled co-delivery of chemotherapeutic agent helps to eradicate tumour growth more than single bullet therapy. At the same time there are new challenges like

- Biocompatibility
- Biostability
- Smooth transfer of drug.

Ideal nano formulation supposed to release its medicament in regulated manner to desired area. Novel methods derived from nano enabled Berberine could deliver better. New strategies based on nano enabled Berberine could be a supplemental process to improved pharmacological action of Berberine as anticancer agent. Along with this further examination of the adjuvant efficiency of Berberine combined with numerous established anticancer will open new door for the addition of Berberine as established anticancer agent.

Furthermore, upcoming research should incorporate estimation of Berberine in upper mammalian animal models of numerous cancer types. These models consist variable profiles & properties compared to rodents & may confirm efficiency of Berberine before transferring to clinical trials. Till date properties of Berberine has been assessed till lab level in last few decades, it is mandatory to investigate more details like

- Molecular target in biological system
- Patient to patient variations
- Dose designs towards personalised medicine.

In today's era of nanotechnology, we need to hold our hands with ancient knowledge to drive safe pharmacotherapy for individual.

Conflict of Interest

The authors declare that there is no conflict of interests regarding this book chapter.

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