Effects of Berberine on Liver Cancer

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Abstract
Liver cancer, otherwise known as hepatocellular carcinoma, is a chronic disease condition with an excessive deposition and growth of malignant cells in the body. The high incidence and prevalence rates of liver cancer continue to be problems, as well as its poor prognosis and therapeutic limitations involving severe drug adverse reactions linked to the use of synthetic chemotherapeutic compounds. Continuous experimental studies, as well as utilization of pure herbal-based compounds, are essential towards finding more potent cures for liver cancer. Natural bioactive compounds, particularly alkaloids (eg, berberine), have been shown to be highly beneficial in the treatment of various diseases. Berberine (BBR), an isoquinoline alkaloid, is obtained from stem, bark, roots, rhizomes, and leaves of several medicinal plants, including Berberis species. It is commonly synthesized from the benzyltetrahydroisoquinoline system with the incorporation of an additional carbon atom as a bridge. The multiple attributes of BBR involving effective inhibitory and cytotoxic actions against the proliferation of cancer cells have been demonstrated. The use of BBR in experimental studies (in vivo and in vitro) for over a decade for liver cancer treatment has proven to be highly effective, safe, and potent. Until now, the poor solubility of BBR remains one of the contributing factors leading to its minimal clinical bioavailability. Therefore, BBR could serve as a prospective drug candidate in the future towards drug formulation for liver cancer treatment. The relevant information regarding this review was obtained electronically through the use of databases such as PubMed, Google Scholar, Springer, Hindawi, Embase, Web of Science, and China National Knowledge Infrastructure. All the aforementioned databases were searched from 1981 to 2020. This literature represents an update of previous review papers discussing the various positive pharmacological and mechanistic effects (oxidative stress regulation, inflammation reduction, apoptosis activation, overcoming drug resistance, and metastasis inhibition) of BBR for liver cancer treatment, which would be of great significance to drug development and clinical research.

Keywords
berberine, liver cancer, apoptosis, epithelial-mesenchymal transition, mechanistic effects

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Introduction
Liver cancer is termed a chronic disease with an excessive or uncontrolled deposition and proliferation of malignant cells in the body.1,2 It is normally accompanied by a manifestation of symptoms like swelling of the abdomen, weakness, weight loss, yellowish skin, and easy bruising and pains in the right side below the rib cage. The occurrence of liver cancer is associated with certain causative agents such as iron overload, obesity, hepatitis-B virus, diabetes, smoking, alcohol-related cirrhosis, liver flukes, hepatitis-C virus, nonalcoholic fatty liver disease (NAFLD), aflatoxin exposure, immunosuppression, and lupus (systemic lupus erythematosus).3-5 The prevalence of liver cancer is still rapidly on the rise worldwide, with a minimal survival rate.6,7 This, combined with poor prognosis and therapeutic shortcomings or limitations involving chronic drug adverse reactions to the synthetic chemotherapeutic...
components applied in drug formulation are causes of concern. The treatments often delay, but can potentiate the degree of disease progression. More experimental studies, as well as utilization of pure herbal-based compounds, are essential towards finding more potent curative agents to treat liver cancer.12

Natural bioactive compounds, particularly alkaloids (eg, Berberine; BBR), have been shown to be highly beneficial in the treatment of various diseases.6–14 BBR is commonly synthesized from the benzyltetrahydroisoquinoline system with the incorporation of an additional carbon atom as a bridge. Current evidence indicates the multiple attributes of BBR involving effective inhibitory and cytotoxic actions (oxidative stress regulation, inflammation reduction, apoptosis, and autophagy activation) against the proliferation of cancer cells.15–21 The use of BBR in experimental studies (in vivo and in vitro) for over a decade against liver cancer has proven to be highly effective, safe, and potent. However, until now, the poor solubility of BBR remains one of the contributing factors leading to its minimal clinical bioavailability. Therefore, BBR could serve as a prospective drug candidate for drug formulation against liver cancer. This work represents an update of previous review papers discussing the various positive pharmacological and mechanistic effects (oxidative stress regulation, inflammation reduction, apoptosis activation, overcoming drug resistance, and metastasis inhibition) of BBR for liver cancer treatment.

Methodology
The information shown in this review was obtained electronically through the use of databases such as PubMed, Google Scholar, Springer, Hindawi, Embase, Web of Science, and China National Knowledge Infrastructure by using keywords such as “Berberine,” “Berberine and Cancer,” “Berberine and Tumor,” “Berberine and Carcinoma,” “Berberine against liver cancer,” “Berberine against hepatocellular carcinoma,” “Hepatocellular carcinoma,” “Liver cancer,” and “Berberine effects on liver cancer.” All the aforementioned databases were searched from 1981 to 2020.

Botanical Sources, Pharmacological Activities, and Metabolism of BBR
The isoquinoline alkaloid, BBR (Figure 1) or 5,6-dihydro-9,10-dimethoxybenzo[g]-1,3-benzodioxolo[5,6-a] quinolizinium derivative, is a constituent of many different medicinal plants such as Berberis species (B. aspiformis, B. vulgaris, B. heterophylla, B. darwinii, B. petiolata, B. buainana, and B. aristata), and others (eg, Hydratis canadensis, Phelodendron chinense, Coptidis sp., Argyonome mexicana, Camellia japonica, Timospora cordifolia, Costinum forestatum, Xanthorrhiza simplicissima, Callosobruchus chinensis, and Phelodendron amurense).22–27 Table 1 lists the BBR containing species (family, botanical names) and their applications. Owing to their yellowish color, Berberis species are commonly utilized in dyeing leather, wood, and wool. BBR also has a wide-ranging history in Asia, being known for its low or poor lipid solubility alongside its remarkable biochemical and pharmacological activities.50–53 Traditionally, BBR, particularly BBR sulfate and hydrochloride, is extensively applied in traditional Chinese and Ayurvedic medicine.54,55

Recent analytical studies have shown that BBR could serve varying purposes, such as anti-obesity, anti-microbial, spatial memory enhancement, anti-diabetic, anti-cancer, anti-atherosclerotic, anti-hypertensive, anti-hyperlipidemic, anti-protozoal, anti-inflammatory, neuroprotective, hepatoprotective, and anti-oxidative agents,56–62 as well as being utilized clinically for the treatment of disease conditions involving polycystic ovary syndrome, NAFLD, CAD, and metabolic syndrome. Table 2 summarizes the numerous beneficial activities of BBR in animal models. The metabolism of BBR is considered mostly to take place in the liver via demethylation (phase I) and glucuronidation processes (phase II), and excreted in the bile.61 Excessive consumption or administration of BBR in large doses (4–6 weeks) results in liver overload. Also, its usage in pregnancy may lead to miscarriage and contraction of the uterus, as well as neonatal jaundice. Other possible toxicological effects or adverse reactions include gastric troubles, headache, digestive problems, stomach upset, constipation, diarrhea, and flatulence.

Potential Utilization of BBR for the Treatment of Liver Cancer
Over the past decade, diverse studies carried out by researchers regarding the medicinal effects of BBR in the treatment of liver cancer cells portrayed or indicated certain clinical outcomes through suppression of cellular proliferation, activation of apoptosis, halting the process of angiogenesis, and slowing down the onset of metastases. In this respect, we outline the prospective in vivo and in vitro actions of BBR in liver cancer (Table 3).

BBR and Liver Cancer
Liver cancer, otherwise known as primary hepatic cancer or hepatocellular carcinoma (HCC), is considered one of the most prevalent and deadliest types of cancer universally, causing deaths with a significant percentage of humans annually.63–66 In 2018, according to World Health Organization
| No | Family            | Botanical name                                      | Application                                                                                                         | References |
|----|-------------------|-----------------------------------------------------|---------------------------------------------------------------------------------------------------------------------|------------|
| 1  | Menispermaceae    | *Tinospora sinensis* (Lour.) Merr (ex *Tinospora cordifolia* (Willd.) Miers) | Utilized as anti-spasmodic, anti-stress, anti-allergic, anti-malarial, tonic, anti-diabetic, anti-arthritis, anti-inflammatory and anti-periodic agents. | 28,29      |
| 2  | Rutaceae          | *Zanthoxylum monophyllum* Tul. *Phellodendron amurense* Rupr. (ex *Phellodendron wilsonii* Hayata & Kaneh.). *Phellodendron chinense* var. *glabriusculum* C. K. Schneid. | Treats dark vomitus and ophthalmic inflammation Used in treating cancer, diarrhea, abdominal pain, gastroenteritis and possesses immunostimulating and anti-inflammatory activities Treats night sweats, vaginal infections, conjunctivitis, skin diseases, jaundice, meningitis, boils, dysentery, acute urinary tract infection, and diarrhea. Serves as vasodilator, aphrodisiac, and expectorant Used as anti-rheumatic and anti-malarial agents, and detoxicant for hot damps related conditions to the kidney. | 30, 31, 32 |
| 3  | Annonaceae        | *Annickia polycarpa* (DC.) Setten & Maas ex I.M.Turner (ex-*Enantia polycarpa* (DC.) Engl. & Diel) | Treats intestinal problems, jaundice, feverish conditions associated with malaria, ophthalmic problems, skin infections, sores, cuts, and improves wound healing. | 32, 33      |
|    |                   | *Annickia chlorantha* (Oliv.) Setten & Maas (ex *Enantia chlorantha* Oliv.) | Treats typhoid and yellow fever, boils, diabetes, vomiting, hypertension, urinary tract infections, malaria, cough, sexual asthma, intestinal worms, tuberculosis, wounds, aches, syphilis, sores, conjunctivitis, jaundice, intestinal spasm, rheumatism, rickettsia fever, prostate cancer, hepatitis A, B, C, and D, dysentery, leishmaniosis, fatigue, sleeping sickness and improves conception. | 32, 34–36   |
|    |                   | *Xylopia polycarpa* (DC.) Oliv. | Promotes conception and utilized in treating malaria, leprosy, sleeping disorders, ulcer, diarrhea, ophthalmic diseases, rheumatism, fever, gall bladder, and stomach problems. | 32          |
|    |                   | *Annickia pilosa* (Exell) Setten & Maas (ex *Enantia pilosa* Exell) | Applied in the treatment of cuts | 32          |
| 4  | Papaveraceae      | *Papaver hybridum* L. | Serve as diuretic, sedative, anti-infective and anti-tussive agents Applied in the treatment of dermatological diseases | 32         |
|    |                   | *Glaucium corniculatum* (L.) Rud. subsp. *corniculatum* | Treats central nervous system disturbances Used as anti-tussive, laxative, and sedative agents | 37, 38      |
|    |                   | *Papaver rhoas* L. var. *chelidoniiodes* | Treats nervous digestive disorders, jaundice, fever, bronchial coughs, insomnia, painful conditions. Applied as sedative, emollient, narcotic, and anticancer agents. | 32          |
|    |                   | *Papaver dubium* L., *Papaver dubium* var. *lecoquii* | Serves as expectorant, ophthalmic, sudorifac, and diuretic agents | 32          |
|    |                   | *Macleaya microcarpa* (Maxim.) Fedde *Macleaya cordata* (Willd.) R.Br. | Treats inflammation and some skin disorders Treats ringworm, and insect bites Utilized as diuretic, analgesic, and anti-edemic agents | 32, 39      |
|    |                   | *Eschscholzia californica* Cham. | Applied in the treatment of insomnia, pain, urinary incontinence, anxiety, spasms, nervous tension, and toothache Promotes perspiration and reduces the flow of milk in lactating mothers. | 32          |
|    |                   | *Bocconia frutescens* L. | Treats respiratory tract infections (tuberculosis and bronchitis), and skin conditions (ulcers) | 32          |
|    |                   | *Argemone platyceras* L. | Used in treating pneumonia, asthma, bronchitis, and cough | 32          |
|    |                   | *Argemone albiflora* Hornem (ex-*Argemone alba* F. Lestib.) | Serves as emetic, purgative and diuretic agents Applied in the treatment of wounds, colds, jaundice, and skin ailments | 32          |
|    |                   | *Argemone mexicana* L. | Utilized as antidote for snake poisoning, sedative, | 32          |

(Continued)
| No | Family | Botanical name | Application | References |
|----|--------|----------------|-------------|------------|
| 1  |        | *Corydalis solida* subsp. *brachylova* | Treats cataract, warts, chronic skin disease, cough, itching, and cold sores. | 40, 41 |
|    |        | *Corydalis solida* subsp. *Slivenensis* (Velen.) Hayek (ex-*Corydalis slivenensis* Velen.); *Corydalis solida* subsp. *taurica*; *Corydalis turtsehaninovii* Besser (ex-*Corydalis ternata* (Nakai) Nakai) | Treats traumatic injury, lumbago, dysmenorrhea, hallucinogenic, rheumatism, cardiac arrhythmia disease, memory dysfunction, duodenal and gastric ulcers; serves as antibacterial, CNS stimulant, antispasmodics, and sedative for insomnia; lowers blood pressure, and calms the nerves. |  |
|    |        | *Chelidonium majus* L. | Treat gastric and duodenal ulcers, memory dysfunction, dysmenorrhea, rheumatism, and cardiac arrhythmia disease. | 32 |
| 2  |        | *Chelidonium majus* L. | Treats traumatic injury, lumbago, dysmenorrhea, hallucinogenic, rheumatism, cardiac arrhythmia disease, memory dysfunction, duodenal and gastric ulcers; serves as antibacterial, CNS stimulant, antispasmodics, and sedative for insomnia; lowers blood pressure, and calms the nerves. |  |
| 3  |        | *Chelidonium majus* L. | Treats traumatic injury, lumbago, dysmenorrhea, hallucinogenic, rheumatism, cardiac arrhythmia disease, memory dysfunction, duodenal and gastric ulcers; serves as antibacterial, CNS stimulant, antispasmodics, and sedative for insomnia; lowers blood pressure, and calms the nerves. |  |
| 4  |        | *Chelidonium majus* L. | Treats traumatic injury, lumbago, dysmenorrhea, hallucinogenic, rheumatism, cardiac arrhythmia disease, memory dysfunction, duodenal and gastric ulcers; serves as antibacterial, CNS stimulant, antispasmodics, and sedative for insomnia; lowers blood pressure, and calms the nerves. |  |
| 5  | Berberidaceae | *Sinopodophyllum hexandrum* (Royle) T. S. Ying | Improves the circulation of blood and modulates menstruation. | 42 |
|    |        | *Caellidophyllum thalictroides* (L.) Michaux | Applied in the treatment of cramps, rheumatism, colic, hysteria, and menstrual cramps. | 32 |
|    |        | *Mahonia napaulensis* DC. | Used in treating eyes-related inflammation, and dysentery. Used as demulcent and diuretics agents. | 32 |
|    |        | *Jeffersonia diphylla* (L.) Pers. | Treats ulcers, sores, urinary problems, and diarrhea. Utilized as emetic, antispasmodic, diuretic, and expectorant in coughs. | 32 |
|    |        | *Nandina domestica* Thunb. | Used in treating fever in influenza, indigestion, acute bronchitis, muscles and traumatic injuries, tooth abscess, whooping cough, and pain in the bones. Serves as anti-rheumatic, astringent, and anti-tussive. | 32 |
|    |        | *Mahonia fortunei* (Lindl.) Fedde | Applied as anti-odontalgic, anticancer, and treating arthritic pain and testicular swelling or inflammation. | 43 |
|    |        | *Berberis aquifolium* Pursh | Utilized in the treatment of jaundice, hemorhaghes, fungal infections, eczema, dysentery, cirrhosis, acne, herpes, hepatitis, sore wound following menstruation or childbirth, psoriasis, digestive problems, conjunctivitis, gall bladder diseases, constipation, stomach problems, skin conditions, and few forms of cancer. Stimulates bile flow, intestinal secretion, and promotes blood flow to the liver. | 44 |
|    |        | *Berberis asiatica* Roxb. ex DC. | Treats ear and eye diseases, rheumatism, hyperpigmentation, headache, diabetes mellitus, malarial fever, asthma, jaundice, toothache, wounds, pneumococcal infections, stomach disorders, inflammation, and ulcers. | 32 |
|    |        | *Berberis actinacantha* Mart. | Anti-pyretic. | 45 |
|    |        | *Berberis pseudumbellata* R. Parker | Treats ulcer, sore throat, jaundice, stomach problems, eye diseases, and intestinal disorders. | 46 |
|    |        | *Berberis vulgaris* L. | Serves as antiarrhythmic, sedative, and anticancer agents. | 47 |
Table 1. Continued.

| No | Family | Botanical name | Application | References |
|----|--------|----------------|-------------|------------|
| 1  |       | Berberis microphylla G. Forst. (ex Berberis heterophylla Juss. ex Poir.) | Used in treating fever, sore throat, internal injuries, and kidney stones | 32 |
| 2  |       | Berberis thunbergii DC. | Applied in the treatment of diarrhea, inflammation, and febrifuge | 48 |
| 3  |       | Berberis oblonga (Regel) C. K. Schneid | Anti-inflammatory | 32 |
| 4  |       | Berberis tinctoria Lesch. | Treats arthralgia, diarrhea, pyrexia, back pain, jaundice, eye diseases, stomach ache, and mouth-related wounds | 32 |
| 5  |       | Berberis petiolata Wall. ex G. Don | Treats jaundice, conjunctivitis, diarrhea, and malarial fever | 49 |
| 6  |       | Berberis umbellata Wall. ex G. Don | Utilized in the treatment of skin problems, eye disorders, fever, nausea, and jaundice | 32 |
| 7  |       | Berberis darwinii Hook. | Treats fever, stomach pains, colitis, and indigestion | 32 |
| 8  |       | Berberis jaeschkeana C. K. Schneid. | Treats eye-related diseases | 32 |
| 9  |       | Berberis lycium Royle | Used in the treatment of skin problems, eye disorders, fever, nausea, and jaundice | 32 |
| 10 |       | Berberis empetrifolia Lam. | Treats cold, complications during post-natal period, and serves as antipyretic | 32 |
| 11 |       | Berberis lycium Royle | Serves as antipyretic | 32 |
| 12 |       | Berberis helianthoides Ehrenb. ex C. K. Schneid. | Treats arthritis, neuralgic diseases, rheumatic, and muscular pain | 32 |
| 13 |       | Berberis integerrima Bunge. | Treats chest pain, diabetes, headaches, bone fractures, constipation, rheumatism, tuberculosis, wound, heart pain, kidney stones, and stomach aches | 32 |
| 14 |       | Berberis leichnhautii Wall. ex Wight & Arn. | Treats cold, complications during post-natal period, and serves as antipyretic | 32 |
| 15 |       | Berberis koroliana Palib. | Serves as antipyretic | 32 |
| 16 |       | Berberis chitra Buch.-Ham. ex-Lindl. | Treats cold, complications during post-natal period, and serves as antipyretic | 32 |
| 17 |       | Berberis aristata DC. |Used in treating infections | 32 |
| 18 |       | Berberis buxifolia Lam. | Treats ophthalmic conditions, skin disease, ulcers, jaundice, rheumatism, ulcers, enlarged liver, and spleen | 32 |
| 19 |       | Berberis buxifolia Lam. | Treats dysentery, osteoporosis, jaundice, urinary tract infections, eye condition, allergies, cholera, metabolic disorders, fever, diarrhea, malaria, skin related diseases, piles, and menorrhagia | 32 |
| 20 | Ranunculaceae | Hydrastis canadensis L. | Used in treating infections | 32 |
| 21 |       | Coptis chinensis Franch. | Treats disorders of the digestive system and mucous membranes, constipation, disorders affecting the ears, eyes, throat, nose, stomach, intestines, and vagina | 32 |
| 22 |       | Coptis teeta Wall. | Control of bacterial and viral infections, relax spasms, and lower fevers | 32 |
| 23 |       | Xanthorrhiza simplicissima Marshall | Treats jaundice, mouth ulcers, digestive disorders, stomach ulcers, piles, and cold | 32 |
| 24 |       | Coptis teeta Wall. | Applied in the treatment of pectoral diseases, ophthalmic conditions, spasms, dysentery, and fevers. Controls bacterial and viral infections, and stimulates circulation | 32 |

(Continued)
(WHO), liver cancer reportedly claimed about 782,000 human lives worldwide. It has greater predominance in males than in females, as well as high regional (Western and Middle Africa, Southeast and East Asia), and low regional (Eastern and Northern Europe, Western and South-Central Asia) incidence rates. Nowadays, lung transplantation, surgery, and radiation therapies provide possible treatments for individuals with early detection. Regardless of the therapeutic advancement, the treatment of liver cancer still remains burdensome due to the tendency of recurrence, even after curative treatment. Varied experimental studies have shown that BBR demonstrates enormous anti-cancer actions.

**Effects of BBR on Overcoming Drug Resistance in Liver Cancer Treatment**

BBR has demonstrated significant characteristics for overcome multidrug resistance, thus exhibiting its capacity in tumor chemotherapy. BBR regulates the neutrophil phenotype to maintain the sensitivity of cancer cells to doxorubicin, synergistically sensitizes human liver cancer cells to sorafenib, and promotes the radio-sensitivity of hepatoma cells by inhibiting the Nrf2 pathway. Also, BBR and the Janus nanocarrier-based co-delivery of doxorubicin impairs chemotherapy-exacerbated HCC recurrence via inhibition of caspase-3-iPLA2-COX-2 signaling pathways. BBR combined with irradiation promotes anti-cancer actions through activation of the p38 MAPK pathway and ROS generation in human hepatoma cells. Additionally, the combination of BBR and vincristine significantly impaired the growth and apoptotic induction in hepatoma cells.

**Effects of BBR on Liver Cancer Metastasis Inhibition**

BBR has been proven to possess positive attributes in the inhibition of tumor metastasis. Matrix metalloproteinases (MMPs) break down the matrix tissue, thereby enabling the malignant cells to advance to the barrier of normal tissues and occupy the surrounding normal tissues and distant organs. BBR suppresses Id-1 expression and inhibits the growth and development of lung metastases in HCC. BBR exerts a strong suppression on the invasion and migration of HCC cells through promoting PAI-1 and decreasing urokinase-type plasminogen activator (uPA).

### Table 1. Continued.

| No | Family | Botanical name | Application | References |
|----|--------|----------------|-------------|------------|
| 6  | Natural Product Communications | *Coptis japonica* (Thunb.) Makino | Treats intestinal catarrh, conjunctivitis, dysentery, high fevers, enteritis, inflamed tongue, and mouth. Stimulates circulation, relaxes spasms, and controls bacterial and viral infections | 32 |

**Mechanistic Effects of BBR on Liver Cancer**

**Oxidative Stress Regulation**

Oxidative stress is a harmful process that can be an essential mediator of either impairment or destruction of cell structures and thus activates different disease conditions, namely cancer, diabetes, and neurological and cardiovascular diseases. Shukia et al demonstrated that BBR regulates oxidative stress through the promotion of reactive oxygen species (ROSs), and lipid peroxidation alongside the down-regulation of the actions of glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) expressions via the JUK signaling pathway. A traditional herbal medicine, *Lagerstroemia speciosa* (L.) Pers., containing of BBR, Gallic acid, and Corosolic acid, induces oxidative stress-mediated apoptosis associated with HepG2 cells through intrinsic and mitochondrial mechanisms.

**Inflammation Reduction**

Inflammation represents a common cause of different chronic diseases. BBR reduces the proliferation of liver cancer through an anti-inflammatory pathway. BBR reduces the protein expression levels of cytosolic phospholipase A2 (cPLA2) and cyclooxygenase (COX)-2, and up-regulates the content ratio of arachidonic acid to prostaglandin E2 in the human hepatocarcinoma (HepG2) cells. BBR suppresses the phosphorylation of Akt, mTOR, and ERK, indicating inhibition of cell growth through the PI3K/Akt/mTOR and ERK/MAPK signal pathways. BBR induces G1-phase arrest accompanied by a decrease in cyclin D1, cyclin E, and cdc2 expressions. It inhibits cell growth through cell cycle arrest. In addition, BBR mediates proliferation and migration in HCC via the Wnt/β-catenin signaling pathway, represses progression or growth of liver cancer cells via inhibiting glutamine uptake, and β-catenin translation involving 4E-binding protein. BBR also exerts anti-proliferative effects against mitochondrial dysfunction-mediated apoptosis in HepG2 cells through down-modulation of the PI3K/Akt/mTOR mechanism, and impairs cell proliferation and migration in HCC via regulation of the Wnt signaling pathway. A traditional herbal remedy, known as DaHuangWan, constituted of BBR and costunolide, suppresses the proliferation of hepatoma cells through modulating the epithelial growth factor (EGF) mechanism. Moreover, BBR can hamper cell proliferation of HepG2, Hep3B, and SNU-182 via promoting protein expression of tumor suppressor genes, like activating transcription factor 3 (ATF3).
Table 2. In Vivo Beneficial Activities Associated With Berberine.

| No | Condition                                      | Details of assay (animal model) | Experimental effects                                                                                                                                                                                                 | Ref. |
|----|------------------------------------------------|---------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| 1  | Intestinal inflammation and gut microbiome     | db/db mice                      | Diminishes body weight, food intake, blood glucose, intestinal inflammation, serum LPS, and HbA1c levels. Restores intestinal SCFA content and barrier structure. Promotes the number of SCFA-producing bacteria and reduces opportunistic pathogens. | 63   |
| 2  | Hypertension                                   | Spontaneously hypertensive rats  | Down-regulates the elevated levels of aldosterone, collagen IV, angiotensin II, collagen III, IL-23, IL-6, IL-17, albumin, osteopontin, and KIM-1.                                                                 | 64   |
| 3  | Diabetic retinopathy                           | STZ induced diabetic retinopathy in male Sprague-Dawley mice | Hinders cell apoptosis, ganglion cell layer, oxidative stress, and deactivates NF-kB signaling pathway                                                                                                                                  | 65   |
| 4  | Diabetic nephropathy                           | HFD and STZ induced diabetic nephropathy rats | Inhibits elevated levels of biochemical indicators, and effectively potentiates the abnormal expression of phosphatidylinositol 3-kinase (PI3K), protein kinase B (Akt), and phosphorylated Akt. | 66   |
|    |                                               | STZ induced diabetic nephropathy rats | Inhibits renal injury, fasting blood glucose, ratio of kidney weight to body weight, 24-h urinary protein, serum creatinine, blood urine nitrogen, systemic and renal cortex inflammatory response, and TLR4/NF-kB pathway. | 67   |
| 5  | Diabetic encephalopathy                        | db/db and C57BL/6j-db/m mice     | Enhances lipid metabolism, synapse and nerve-related protein expression (NGF, SYN, and PSD95), and protein expression of SIRT1. Downregulates fasting blood glucose, protein expression of inflammatory factors (NF-kB and TNF-α), and ER stress-associated proteins (IRE-1α, CHOP, PDI, PERK, and eIF-2α). | 68   |
| 6  | Axonopathy and diabetic encephalopathy         | HFD and STZ induced diabetic rats | Decreases blood glucose, tau hyperphosphorylation, axonopathy, memory impairment, insulin level, insulin resistance, and restores PI3K/Akt/GSK3β signaling pathway                                                                 | 69   |
| 7  | Diabetes                                       | STZ induced diabetic albino Wistar mice | Significantly suppresses hepatic markers, lipid peroxidation markers (LOOH and TBARS), pro-inflammatory mediators (TNF-α, phospho-NF-kB p65, COX-2, and iNOS), and pro-apoptotic mediators (Bax and cytochrome c). Potentiates hexokinase, glucose-6-phosphate dehydrogenase, enzymatic antioxidant (SOD, CAT, and GPx), non-enzymatic antioxidants (GSH, vitamin E, and vitamin C), and anti-apoptotic protein (Bcl). | 70,71|
|    |                                               | Alloxan and HFD induced diabetic Wistar mice | Represses the fasting blood glucose level, serum content of LDL-c, TG, and TC. Improves HDL-c, NO, SOD, and GSH-px. Obstructs the increase of MDA and restored the damage to pancreas tissues.                                                                 | 72   |
|    |                                               | HFD and STZ induced diabetic mice | Down-regulates the levels of fasting blood glucose, LDL-c, total cholesterol, hypothalamic orexin-A, OX2R receptor, corticotropin-releasing hormone, pituitary and plasma adrenocorticotropic hormone, serum and urine corticosterone. Promotes the insulin sensitivity index, abnormalities of HDL-c, insulin resistance index, insulin levels, glucagon, mRNA, and protein expressions of GLUT4 in skeletal muscles. | 73   |
| 8  | Alzheimer’s disease and type-2 diabetes mellitus | STZ and Aβ25–35 induced Alzheimer’s diabetic mice | Attenuates memory deficits, ER stress, and the increased levels of triglyceride, total cholesterol, fasting blood glucose, and glycosylated serum protein. Restores the disordered arrangements of nerve cells and up-regulates TUNEL-positive cells. | 74   |
| 9  | Alzheimer’s disease                            | APP/PS1 transgenic rats          | Enhances learning and memory, and glutathione (GSH) activity. Reduces hyperphosphorylated tau protein, lipid peroxidation, and NF-kB activity.                                                                                                                                         | 75   |
|    |                                               | APP/PS1 transgenic rats          | Inhibits the levels of Aβ, sAPP-β, BACE1, PS1, Pen-2, and Aph-1α. Promotes the levels of ADAM17, sAPPα, ADAM10, learning, and memory.                                                                                                                                  | 76   |
| 10 | Anxiety                                       | Male Wistar mice                | Alleviates locomotor activity, relapse, and anxiety-related behaviors. Increases TLR4, Sirt1, and α-actin activation.                                                                                                                                                               | 77   |
| 11 | Ulcerative colitis                             | DSS-induced mice                | Diminishes the expression of IL-12, IL-6, IFN-γ, IL-1, IL-1β,                                                                                                                                                                                                                | 78   |

(Continued)
Table 2. Continued.

| No | Condition                  | Details of assay (animal model)                      | Experimental effects                                                                 | Ref. |
|----|----------------------------|-----------------------------------------------------|----------------------------------------------------------------------------------------|------|
| 12 | Myocardial infarction     | Male wild-type (C57BL6) rats                        | TNF-α, MPO, iNOS, MDA and TGF-β. Up-regulates the expression of IL-10, IL-4 and SlgA.  | 79   |
|    |                            |                                                     | Promotes miR-29b expression level, angiogenesis, and heart functions. Reduces infarct size. |      |
| 13 | Myocardial hypertrophy    | CAA induced myocardial hypertrophy mice             | Decreases the expression of β myosin heavy chain, atrial natriuretic peptide, and myocardial infarction associated transcript. Halts up-regulation of mRNA expression and downregulation of bedlin 1 and autophagy-related 5. | 80   |

Table 3. Mechanistic Actions of Berberine on Liver Cancer.

| No | Condition                  | Assay model (animal/cell/tissues)                   | Experimental effects                                                                 | Ref. |
|----|----------------------------|-----------------------------------------------------|----------------------------------------------------------------------------------------|------|
| 1  | Liver cancer               | SMMC-7721 and HepG2 cells                          | Up-regulates the expression levels of cleaved caspase-3 and cleaved poly (ADP-ribose) polymerase, and reduces vascular endothelial growth factor and anti-apoptotic protein B-cell lymphoma. 2 | 82   |
|    |                            | Huh7 and HepG2 cells                                | Down-regulates the expression of Nrf2 signaling-related protein (Nrf2, HO-1, and NQO-1). Potentiates the radiation-induced oxidative stress and apoptosis. | 83   |
|    |                            | Human hepatoma HepG2 cell                           | Potentiates the levels of nitric oxide and reactive oxygen species                     | 84   |
|    |                            | MHCC-97L cell                                       | Decreases cellular proliferation, invasiveness, and HIF-1α/VEGF signaling pathway. Decrease the transcription level of Id-1 via suppressing its promoter activity. | 85   |
|    |                            | Bel-7402 and SMMC-7721 cells                        | Decreases the expression of cyclooxygenase-2 (COX-2), nuclear factor kappa B (NF-kB), urokinase-type plasminogen activator, and matrix metalloproteinase-9 (MMP-9). Inactivates p38 and Erk1/2 signaling pathway. | 86   |
|    |                            | Human hepatocarcinoma (HepG2) cell                  | Increases the mRNA expression of FoxO1 and FoxO3a. Improves JNK phosphorylation, ROS generation, and lipid peroxidation. Reduces level of catalase, superoxide dismutase, and glutathione. | 87   |
|    |                            | H22, HepG2, and Bel-7404 cells                      | Reduces the protein expression levels of cytosolic phospholipase A2 (cPLA2) and cyclooxygenase (COX)-2. Up-regulates the content ratio of arachidonic acid to prostaglandin E2. | 88   |
|    |                            | HepG2, Bel-7402, and SMMC-7721                      | Hampers the expression of cyclin D1, cyclin E, and cdc 2 and reduces the phosphorylation of Akt, mTOR, and ERK. | 89   |
|    |                            | BALB/c nude mice, Hep3B, and BEL-7404 cells         | Suppresses the growth of HCC cells in vitro. Inhibits the glutamine uptake by diminishing SLC1A5. Decreases the proliferation of tumor xenografts, and the expression of SLC1A5 and c-Myc in-vivo. | 90   |
|    |                            | Hep3B, HepG2, HEK293, and Huh7 cells                | Modulates β-catechin pathway independent of AMPK. Antagonizes β-catechin pathway via targeting Cap-dependent translation. Regulates β-catechin expression at the level of translation. Inhibits mTOR pathway and Cap-dependent translation. Activates HCC cell apoptosis via antagonizing Cap-dependent translation and β-catechin axis. | 91   |
|    |                            | Human hepatocarcinoma (HepG2) cell                  | Triggers the activation of caspase-8 and caspase-3, release of cytochrome c and PARP (poly ADP-ribose polymerase) cleavage, and down-regulates expression of Bid and anti-apoptosis factor BelXL. | 92   |
|    |                            | HepG2, SMMC-7721, and Bel-7402 cell                | Significantly elevates phosphorylated AMP-activated protein kinase, phosphorylated Akt level, and Bax/Bcl-2 ratio in a dose-dependent manner. | 93   |
|    |                            | Human hepatoma WRL68 cells                          | Improves the expression of Bax. Regulates the protein expression of Bcl-2 associated with caspase -3/7 activities. | 94   |
|    |                            | Human hepatoma HepG2 cell                           | Diminishes the expressions of Bcl-2 protein and pro-caspase-3, and promotes Bax protein. | 95   |
|    |                            | Human hepatocarcinoma (HepG2) cell                  | Down-regulates the NF-kB p65 level. | 96   |
|    |                            | Human hepatocarcinoma (HepG2) cell                  | Reverses the adhesion and migration of HepG2 cells via suppressing the expression of LOX-5 and decreasing the LTβ4 production in the tumor microenvironment. | 97   |
|    |                            | HepG2 and MHCC97-L cells                            | Enhances Bax expression, formation of permeable transition pores, cytochrome C release to cytosol, and activation of the caspases 3 and 9 execution pathway. | 98   |
Apoptosis Activation
BBR potentiates the mitochondria-dependent pathway to activate apoptosis in human hepatocarcinoma (HepG2) cells. BBR restores the activation of caspase-3 and caspase-8 and releases mitochondrial membrane potential, cytochrome c, and cleavage of poly ADP-ribose polymerase (PARP), resulting in a decrease in the expression level of Bid and anti-apoptosis factor Bcl-XL. BBR remarkably activates the mRNA level or expression of FoxO1 and FoxO3 and inhibits their breakdown. Hence, BBR promotes the transcriptional effect of FoxO that is related to the anti-proliferation of tumors. Elevating FoxO transcriptional factors vigorously activates the level of the BH3-only protein Bim and induces the pro-apoptotic protein Bax and caspases, leading to mitochondria-mediated apoptosis. In addition, BBR activates apoptosis in liver cancer cells via inhibition of the AMPK-mediated mitochondrial/caspase pathway, Akt-ASK1-P38MAPKs linked cascade, NF-kB p65 pathway, and the iPLA2/LOX-5/LTB4 signaling pathway. The combined use of BBR and evodiamine also improves the apoptosis of human HCC SMMC-7721 cells. Furthermore, the use of Berberis lycium Royle in the apoptotic treatment of HepG2 cells, revealed a decrease in Bcl-2, independent of p53 Mrna, and an increase in CDK1 while suppressing CDK5, CDK9, and CDK10 mRNA expressions.

Autophagy
Autophagy is described as a natural, conserved catabolic pathway, through which eukaryotic cells degrade or recycle internal components via a membrane trafficking mechanism. It also serves as a source of sustainable energy and biomolecules to the cells for proper maintenance of intracellular homeostasis during stressful conditions like a tumor microenvironment.

BBR activates autophagic cell death in HepG2 and MHCC97-L cells through suppression of the mTOR mechanism and Beclin-1 activation by increasing P38 MAPK and decreasing the activities of Akt signaling. BBR also induces the glucose-regulated protein 78 (GRP78) level via down-regulation of proteasomal and ubiquitination degradation, and activation of ATF6 cleavage, thereby resulting in cancer cell death and autophagy. BBR, obtained from Coptidis Rhizoma, triggered autophagic cell death via suppression of the PI3K/Akt/mTOR mechanism and elevation of ROS-mediated mitochondrial dysfunction in HCC Hep3B cells. In addition, BBR sensitized human HCC to ionizing radiation by obstructing cell cycle arrest and autophagy, resulting in senescence, triggered autophagic and apoptotic death in HepG2 cells through the activation of AMPK mechanism, and activated cell death in human hepatoma carcinoma cell line HepG2 through inhibiting the expression of CD147. Finally, BBR suppressed the viability, migration, and invasion capacity of HepG2.
### Table 4. Formulation Techniques Designed to Promote the Bioavailability and Effectiveness of Berberine as an Anti-Cancer Agent.

| No | Preparation Attributes | Experimental model and effects | Ref |
|----|-------------------------|-------------------------------|----|
| 1  | Nano-sized carbon nanocarrier-C₆₀ fulleren (C₆₀) | Water dispersions of noncovalent C₆₀-Ber nano-complexes in 1:2, 1:1, and 2:1 molar ratios | Improves the intracellular uptake of BBR; higher anti-proliferative potential towards CCRF-CEM cells free—Berberine < 1:2 < 1:1 < 2:1 molar ratio preparations; induce caspase 3/7; cell cycle arrest at sub-G1 phase; activate apoptosis. |
| 2  | Cationic and anionic vitamin E-TPGS mixed polymeric phospholipid micellar vehicles | Lipid-based nanoparticles, amphiphilic mixed micelles composed of polymeric phospholipid conjugates and PEG-succinate ester of tocopherol. | Human prostate cancer cell lines (PC3 and LNPc) promote apoptosis activation with a 30-fold potential improvement of pharmacokinetics. |
| 3  | Novel mitochondria targeting surface charge-reversal polymeric nanocarrier | Vitamin B6-oligomeric hyaluronic acid (OHA)-dithiodipropionic acid-BBR preparation; BBR conjugated with OHA and OHA further conjugated to B6. Micelles of 172.9 nm are formed by formulating conjugates with Cur-loaded nanoparticles. | Triggers cytotoxicity in vitro against PANC-1 cells and tumor proliferation in nude rats bearing PANC-1 cells xenograft; subcellular drug distribution indicates mitochondria as a target. |
| 4  | Planar side arm-tethered β-cyclodextrin encapsulation | Fluorenyl derivative of β-cyclodextrin used to encapsulate BBR. | Actively binds with duplex and G-quadruplex DNAs although its association with the cavity of β-cyclodextrin reduces the strength of binding. |
| 5  | Cationic γ-cyclodextrin derivative | A cationic derivative of γ-cyclodextrin synthesized via modification with propylenediamine; mucoadhesive with resistance to digestion by α-amylase. | Localized in lysosomes with cytotoxicity twice higher than BBR in murine melanoma (B16-F10) and 4T1 cells. |
| 6  | PLGA nanocarrier | PLGA-doxorubicin conjugate utilized in BBR encapsulation. | Anti-proliferative against MDA-MB-231 and T47D breast cancer cell lines were observed with IC₅₀ of 1.94 ± 0.22 and 1.02 ± 0.36 μM; changes mitochondrial permeability and arrest cell cycle at sub G1 phase; 14-fold up-regulates in the half-life of BBR in rats. |
| 7  | Self-carried berberine microrods | Particles prepared through mixing trimethylamine with BBR hydrochloride in DMSO to form about 20–100 μm in length and 5–20 μm width irregular size product. | With about 40 μg/mL IC₅₀ value, about twice more selective than BBR in cancer cells. |
| 8  | Polyethyleneimine (PEI)-cholesterol (PC) berberine nanocarrier complexed with miR-122 | BBR incorporated to PC with further electrostatic complex with miR-122; good drug loading (8.4%) and release (63.0) capacity of nanoparticles of about 146 nm. | Downregulates OSCC cells invasion and migration in transwell studies when compared with single-drug therapeutics. |
| 9  | BBR with PEGylated liposomal doxorubicin (PEG-lip-DOX) | BBR merged with polyethylene glycolated liposomal doxorubicin. | Suppresses the vascular endothelial growth factor (VEGF) expression in human umbilical vein endothelial cells (HUVECs); reduces (via i.v.) tumor proliferation in Meth A sarcoma-transplanted mice; effect stronger than BBR or PEG-lip-DOX alone. |
| 10 | Zinc oxide-based nanocarrier | BBR and zinc oxide (ZnO) combined through facile blending at the ratio of 39:61 to form 200–300 nm size nanoparticles. | Promotes anti-growth activity in A549 (human lung adenocarcinoma) cells; no obvious severe hepatotoxicity, hemotoxicity, and renal toxicity in rats by i.v. |
| 11 | Folic acid- and BBR-loaded silver nanomaterial (FA-PEG@BBR-AgNPs) | Encapsulating BBR on citrate-capped silver nanoparticles (AgNPs) through electrostatic interactions (BBR-AgNPs) followed through conjugation with polyethylene glycol-functionalized folic acid by hydrogen bonding interactions | Improves apoptosis in MDA-MB-231 breast cancer cells; activates ROS; regulates P3K, AKT, Ras, Raf, ERK, VEGF, HIF1α, Bel-2, Bax, cytochrome-c, caspase-9, and caspase-3; triggers tumor proliferation in vivo when (Continued)
HepG2 cells via the activation of pyroptosis (caspase-1 dependent programmed cell death) both in vitro and in vivo, which was impaired by caspase-1 inhibitor Ac-YVAD-CMK.28

In summary, BBR prevents cellular growth, proliferation, migration, autophagy, apoptosis, and cell cycle arrest processes in HCC cells via the inhibition of AMPK-mediated mitochondrial/caspase, arachidonic acid metabolic, NF-κB-mediated mitochon-
drional/nuclear pathways. Figure 2 indicates the schematic illustration of BBR effects and mechanisms on liver cancer.

The Enhancement of BBR Effects Through New Drug Formulation

Table 4 shows several formulation techniques particularly designed for tackling the limitations (poor intestinal absorption, poor pharmacokinetic, or poor bioavailability) associated with BBR administration, thereby promoting its anti-cancer efficacy.29–45 These involve nanocarriers or nanoparticles of different surface charges and size with few targeting subcellular organelles like mitochondria. For instance, the experimental studies carried out by Khan et al, demonstrated a 14-fold elevation in the half-life of BBR in a mice model through poly (lactic-co-glycolic acid) (PLGA) nanoparticle BBR carriers, while the charged vitamin E-based amphiphilic mixed micellar vehicles provided a 30-fold enhancement in BBR pharmacokinetics in mice with about 2-fold advancement in half-life ($t_{1/2}$).

Conclusion and Future Perspectives

In this review, we aimed to collect all information with regards to the various positive pharmacological and mechanistic effects
of BBR for liver cancer treatment. The highlighted in vivo and in vitro experimental studies carried out for over a decade with the use of BBR for liver cancer treatment has proven the compound to be highly effective, safe, and a potent natural product, which could serve as a prospective choice as an herbal remedy in treating liver cancer. It also known to possess a wide spectrum of clinical usage against various diseases, such as polycystic ovary syndrome, NAFLD, CAD, and metabolic syndrome. However, a significant drawback is its hydrophilicity, known to be associated with the use of BBR in malignant or tumor treatment, thereby leading to poor bioavailability and low effective concentration. However, it has been proven that complexes of iron-oxide nanoparticles and the hypoxic cell sensitizer sanazole, in conjunction with BBR, represent a highly efficient improvement in the therapeutic specificity and bioavailability of BBR. However, more studies are required regarding the activities of BBR in preventing cellular growth, proliferation, migration, autophagy, apoptosis, and cell cycle arrest processes in HCC cells via different signaling pathways or mechanisms, so as to provide extensive analytical data to aid the conduction of clinical research in the future.

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