Anticancer Potential of Natural Isoquinoline Alkaloid Berberine

Ganesh C. Jagetia

Department of Zoology, Mizoram University, Aizawl-796004, Mizoram, India

Received: March 03, 2021 | Revised: April 12, 2021 | Accepted: April 14, 2021 | Published: Month 00, 2021

Abstract

Despite the availability of several therapeutic strategies and many drugs, the ability to cure most cancers remains a challenge. Natural products have been used for the treatment of numerous diseases, including cancer. The present review delineates various preclinical studies performed in vitro and in vivo that explore the anticancer potential of berberine, an isoquinoline alkaloid found in numerous plants as a secondary metabolite. Berberine can kill various types of human cancer cells in an optimal concentration- and duration-dependent manner and inhibit the growth of various types of cancers in animal models by elevating oxidative stress. In addition, berberine suppresses cell migration, invasion and epithelial-to-mesenchymal transition in different types of cancer cells. Mechanistically, berberine can induce cancer cell DNA fragmentation/apoptosis through extrinsic and intrinsic pathways, autophagy and necrosis. The cytotoxic effects of berberine in different types of cancer cells are mediated by its ability to induce oxidative stress and cell cycle arrest, and inhibit cell migration, invasion and epithelial-to-mesenchymal transition as well as matrix metalloproteinases through the modulation of Wnt and β-catenin signaling. A single clinical study has shown some promise in gastric cancer patients. Though berberine is a relatively safe compound, it should not be prescribed to pregnant or lactating women to avoid adverse effects on developing fetuses and neonates.

Keywords: Berberine; Apoptosis; Cylins; Reactive oxygen species; beta-catenin; Caspase.

Abbreviations: ACC, acetyl-CoA carboxylase; ACL, ATP citrate lyase; AIF, apoptosis inducing factor; AMPK, AMP-activated protein kinase; AP, activator protein; Apaf, apoptotic protease activating factor; ATF, activating transcription factor; ATX, atraxia telangiectasia mutated; ATP, adenosine triphosphate; Bad, Bcl-2 antagonist/killer; Bas, BCL2-associated X apoptosis regulator; Bcl, B-cell lymphoma; BID, BH3 interacting domain death agonist; BMP, bone morphogenetic protein; C/EBP, CCAAT-enhancer-binding protein; CCR, C-C chemokine receptor; cdc, cell division cycle; CDK, cyclin-dependent kinase; CDKIs, cyclin-dependent kinase inhibitors; COX, cyclooxygenase; CXCR, C-X-C motif chemokine receptor; DDIG, DNA damage-inducible gene; DSBs, double-strand breaks; EBNA1, Epstein–Barr nuclear antigen 1; EGFR, epidermal growth factor receptor; EMT, epithelial-to-mesenchymal transition; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; ETIF, eukaryotic translation initiation factor; FADD, FAS-associated death domain; FAK, focal adhesion kinase; FASN, fatty acid synthase; FoxO3a, forkhead box O3a; GADD, growth arrest and DNA damage-inducible genes; GIP, glucose-regulated protein; GSH, glutathione; GSK, glycogen synthase kinase; HDAC, histone deacetylase; hERG, human ether-à-go-go-related gene; HIF, hypoxia inducible factor; IIHCBP, immunoglobulin heavy chain binding protein; IL, interleukin; JNK, c-Jun N-terminal kinase; LC3, microtubule associated proteins 1A/1B light chain 3B; LDL, lactate dehydrogenase; MEK/ERK, mitogen-activated protein kinase; MMP, matrix metalloproteinases; MRB, mitochondrial ribosomal protein; mTOR, mammalian target of rapamycin; NADPH, nicotinamide adenine dinucleotide phosphate hydrogen; NAG, nonsteroidal anti-inflammatory drug activated gene; NAT, N-acetyltransferase; NCAM, neural cell adhesion molecule; Nestin, neuroectodermal stem cell marker; NF-κB, nuclear factor kappa B; Notch, neurogenic locus notch homolog protein; PARP, poly(ADP-ribose) polymerase; PCNA, proliferating cell nuclear antigen; PTCD, pentatricopeptide repeat domain; PTEN, phosphatase and tensin tumor suppressing gene; RAF, rapidly accelerated fibrosarcoma; Ras, retrovirus-associated DNA sequences; ROS, reactive oxygen species; SCAP, SREBP cleavage-activating protein; Sipk, S-phase kinase-associated protein; SQSTM1, sequestosome-1; SREBP, sterol regulatory element-binding protein; STAT, signal transducer and activator of transcription; TF, T-cell factor; TGF, transforming growth factor; TIP, translation initiation factor; TRAIL, tumor necrosis factor-(TNF) related apoptosis-inducing ligand; TUM, To translation elongation factor; ULC, UNC5-like autophagy activating kinase; VASP, vasodilator-stimulated phosphoprotein; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; Wt, Wnt inhibitory factor; WTX, Wilms tumor gene on X chromosome; XIAP, X-linked inhibitor of apoptosis protein; Δσm, mitochondrial membrane potential.

Correspondence to: Ganesh C. Jagetia, 10 Maharana Pratap Colony, Sector 13, Hiran Magri, Udaiapur 313002, India. ORCID: http://orcid.org/0000-0002-4514-2569. Tel: +919456352849, E-mail: gc.jagetia@gmail.com

How to cite this article: Jagetia GC. Anticancer Potential of Natural Isoquinoline Alkaloid Berberine. J Explor Res Pharmacol 2021;00(00):00-00. doi: 10.14218/ JERP.2021.00005.
10 million in the year 2020. The availability of state of art treatment regimens in modern therapy has not significantly reduced the cancer burden in society. The cost of cancer treatment has seen a phenomenal increase in recent years due to the approval of high-cost oncology drugs and other related expenditures. Modern cancer treatment regimens put a heavy economic burden on the families of cancer patients as it drains the majority of their financial resources, and the cost of cancer treatment will still see an upward trend moving forward. Therefore, it is necessary to search for new cost-effective chemotherapeutic agents with fewer toxic implications.

Many modern cancer chemotherapeutic drugs were initially derived from natural resources before the chemical synthesis was undertaken. Natural products may be a great resource to aid in the search for novel cancer treatments as they may be more economic than high-cost exotic chemotherapeutic drugs. Moreover, they may also overcome the drug resistance induced by modern chemotherapeutic agents, which is a major cause of treatment failure. Berberine is a natural isoquinoline alkaloid synthesized by numerous plants including goldenseal (Hydrastis canadensis), yellowroot (Phellodendron amurense), Chinese goldthread (Rhzona coptidis), Oregon grape (Berberis aquifolium), goldthread or savoyane (Coptis groenlandica), Indian goldthread (Coptis teeta), Indian barberry (Berberis aristata), bayberry (Berberis vulgaris), barberry (Berberis napalensis), Baical skullcap root (Radix scutellariae), Amur cork tree (Coptis chinensis), tree turmeric (Berberis aristata), giloe (Tinospora cordifolia), Californian poppy (Eschscholzia californica), Lopez root or Forest pepper or wild orange tree (Fodellia aculeata), false calumba (Coscinium fenestratum) and prickly poppy (Argemone mexicana).

Berberine (natural yellow 18), also known as 7,8,13,13a-tetradehydro-9,10-dimethoxy-2,3-(methylenedioxy) berbiniun or 5,6-dihydro-9,10-dimethoxybenzog(g)-1,3-benzodioxolo(5,6-a) quinolinizinium, is an isoquinoline alkaloid with a molecular weight of 336.367 g/mol (Fig. 1). Berberine chloride is soluble in warm water at a concentration of 3.2 mg/mL, but its solubility is less in cold water (2 mg/mL). The solubility in organic solvents is 75 mg/mL in DMSO, greater than 50 mg/mL in methanol, and greater than 2 mg/mL in ethanol; however, it is sparingly soluble in chloroform. Berberine belongs to the family of protoberberine alkaloids. It is a bright yellow fluorescent powder, which has been used in India and other countries to dye wool, wood, and leather. Berberine possesses yellow fluorescence under ultraviolet light and it is also used as a stain for histological examinations.

Berberine-containing plants have been used in traditional Ayurvedic and Chinese systems of medicine to treat various disorders in humans for a long time. Berberine acts as an antimicrobial, anti-oxidant, antibacterial, anti-inflammatory, anti-tumor, antidepressant, antidiabetic, antihypertensive, anti-arhythmic, anti-osteoarthritic, chemo-sensitizing, hepatoprotective, and neuroprotective agent. It is active against ischemia-reperfusion injury, and clinical trials have shown that berberine can control dyslipidemia, dementia, ocular Behçet’s disease, hyperlipidemia, and non-fatty liver disease. The focus of this review will be to delineate the anticancer activities of berberine alone in vitro and in vivo.

**In vitro studies**

The anticancer potential of berberine has been studied in vitro using numerous neoplastic cell lines of different tissue origins with specific studies detailed below (Table 1).

### Brain cancer

Treatment of human glioblastoma T98G cells with 50, 75, 100, 150, and 200 µg/mL berberine reduced cell proliferation and increased cell death in a concentration-dependent manner with an IC₅₀ of 134 µg/mL. Berberine arrested cells in the G₁ phase of the cell cycle, owing to a rise in p27 and decline in cyclin-dependent kinase (CDK) 2/4 and cyclin D/E (Table 1). Berberine triggered apoptosis in T98G cells by elevating the Bcl-2-associated X (Bax)/B cell lymphoma 2 (Bcl-2) protein ratio, in addition to procaspase-9, caspase 9/3, and poly(ADP-ribose) polymerase (PARP), and by disrupting the mitochondrial membrane potential (ΔΨm). T98G cells treated with berberine had increased reactive oxygen species (ROS), intracellular Ca²⁺ generation, phosphorylation of endoplasmic reticulum (ER) stress-associated ER kinase, eukaryotic translation initiation factor-2α (ETIF-2α), glucose-regulated protein (GRP) 78, immunoglobulin heavy chain binding protein (IHCBP), CCAAT/enhancer-binding protein (C/EBP)-homologous protein, growth arrest and DNA damage-inducible gene 153 (GADD153), and activation of caspase 3, 8, 9.

Treatment of C6 rat glioma cells with 50, 100, 200, and 500 µM berberine stimulated morphological changes and increased apoptosis (Table 1). Berberine upregulated the expression of Weel and suppressed cyclin B, CDK1, and cell division cycle (Cdc)2/c3, thereby arresting the cells in the G₂/M phase of the cell cycle. Berberine triggered mitochondrial cytochrome c release and elevated caspase 9/3/8, DNA fragmentation, Bax, GADD153 and GRP78, but suppressed Bcl-2 and reduced ΔΨm.

Treatment of human glioblastoma U87, U251, and U118 cells with 15, 25, 50, 100, and 150 µM berberine reduced cell viability depending on the length of treatment time and drug concentration, and the IC₅₀ values were 21.76, 9.79, and 35.54 µM, respectively. Berberine increased senescence in U87 cells, and in U251 cells (lacking PTEN) up to day seven when the S-phase cells were minimal (Table 1). Berberine elevated DNA double-strand breaks (DSBs) indicated by a rise in phosphorylated H2A histone family member X (γ-H2AX) in U251 cells but not in U87 cells. Berberine reduced the expression of epidermal growth factor receptor (EGFR) as well as the phosphorylation of RAF, mitogen-activated protein kinase kinase (MEK), and extracellular signal-regulated kinase (ERK) in U87 and U251 cells. Exposure of U87, U251, and P3 human glioma and astrocyte cells to 50, 100, 150, 200, and 250 µM berberine decreased cell proliferation in a concentration-dependent manner. Berberine arrested cells in the G1 phase of the cell cycle, owing to a rise in p27 and decline in cyclin-dependent kinase (CDK) 2/4 and cyclin D/E. Berberine triggered apoptosis in T98G cells by elevating the Bcl-2-associated X (Bax)/B cell lymphoma 2 (Bcl-2) protein ratio, in addition to procaspase-9, caspase 9/3, and poly(ADP-ribose) polymerase (PARP), and by disrupting the mitochondrial membrane potential (ΔΨm). T98G cells treated with berberine had increased reactive oxygen species (ROS), intracellular Ca²⁺ generation, phosphorylation of endoplasmic reticulum (ER) stress-associated ER kinase, eukaryotic translation initiation factor-2α (ETIF-2α), glucose-regulated protein (GRP) 78, immunoglobulin heavy chain binding protein (IHCBP), CCAAT/enhancer-binding protein (C/EBP)-homologous protein, growth arrest and DNA damage-inducible gene 153 (GADD153), and activation of caspase 3, 8, 9. The anticancer potential of berberine has been studied in vitro using numerous neoplastic cell lines of different tissue origins with specific studies detailed below (Table 1).

### In vitro studies

The anticancer potential of berberine has been studied in vitro using numerous neoplastic cell lines of different tissue origins with specific studies detailed below (Table 1).
### Table 1. Anticancer activity of berberine in various cultured cell lines and its mechanism of action

| Cell line/IC$_{50}$ | Berberine concentration | Outcome | Mechanism | References |
|----------------------|-------------------------|---------|------------|------------|
| Neuroblastoma        |                         |         |            |            |
| T98G/134 µg/mL        | 50, 75, 100, 150, or 200 µg/mL | Decreased cell proliferation, increased cell death, ER stress, apoptosis | G$_s$ arrest, increased p27; reduced CDK2/4, cyclin D1,E; increased Bax, procaspase-9, caspase-3, and PARP; ROS; Ca$^{2+}$, ER kinase, ETIF-2a, GLP78, C/EBP, DDG153, disrupted Δψm | 30,31 |
| C6 (rat)              | 50, 100, 200, or 500 µM | Increased cell death and apoptosis, DNA fragmentation, ER stress, G$_s$/M arrest | Increased Wee1, cytochrome c, caspase-9/3/8, Bax, GADD153,GRP 78, decreased cyclin B, CDK1, Cdc25c, Bcl-2, Δψm | 32 |
| U87/21.76; U25/19.79; U118/35.54 µmol/L; P3 | 15, 25, 50, 100, or 150 µmol/L; 50, 100, 150, 200, or 250 µM | Decreased cell viability, cell proliferation, migration, invasion, EMT, increased senescence, cell death, apoptosis, autophagy | Increased DNA DSBs, Bax, cytochrome c release, caspase 3, LC3B-II, AMPK, ULK-1, Beclin-1, oxidative phosphorylation, SOX11/P62, decreased IL-18, IL-1β, EGFR, RAF, MEK, ERK1/2, Bcl-2, L-lactate, LDH | 33–35 |
| Na2 (mouse); IMR-32  | 10 or 20 µg/mL | Decreased cell proliferation, EMT increased cell differentiation, cell cycle arrest | Decreased CD133, β-catenin, n-myc, sox2, notch2, nestin, CDK-2, CDK-4, cyclin D1/E, P13/Akt, Ras-Raf-ERK, increased, p27, p53, NCAM, laminin, Smad, Hsp70, p38-ATP MAPK | 36 |
| T98G, LN18; LN229, C6, SHG 44 | 25, 50, 100, 200, or 400 mg/L | Decreased cell viability, oxygen consumption rate, mitochondrial respiration, increased autophagy, apoptosis, necrosis | Decreased ATP, GSH, NADPH, aerobic oxidation, p-ERK1/2, increased aerobic glycolysis | 37 |
| LN229/40 µM; U251/30 µM | 5, 10, 20, 40, 80, 160, or 320 µM | Decreased cell proliferation, increased apoptosis | Increased Wifi-1, decreased Bcl-2, Wnt/β-catenin signaling, β-catenin/TCP-4 transcription | 38 |
| U87MG                 | 10, 25, 100, or 250 µM | Decreased cell viability, cell proliferation, increased apoptosis | Decreased MMP-2/9, u-PA, FAK, pJNK, pERK, IKK, NF-κB, Bcl-2, Bcl-xL, Δψm, increased ROS, Ca$^{2+}$, cytochrome c, Bax, Bad, caspase 8/9/3, Apaf1, Fas, FADD, AIF, EndoG | 39 |
| Head and neck cancer  |                         |         |            |            |
| KB                   | 1, 10, or 100 µM; 0.01, 0.1 or 1 µg/mL | Decreased cell viability, cell migration, increased apoptosis, DNA fragmentation, cell cycle arrest | Increased caspase 3/7/8/9, PARP, FasL, Bax, Bad, Apaf-1, decreased COX-2, Mcl-1, Akt, Ras-Raf-ERK, increased, p27, p53, NCAM, laminin, Smad, Hsp70, p38-ATP MAPK | 40,41 |
| HSC-3                | 5, 10, 25, 50 or 75 µM | Decreased cell growth, DNA synthesis, increased apoptosis, G$_s$/G$_1$ arrest | Increased ROS, Ca$^{2+}$, p53, cytochrome c, decreased Bcl2 | 42 |
| SCC-4                | 65.2 or 125 µM | Decreased cell viability, cell migration, invasion, increased DNA damage, apoptosis | Decreased MMP-2/9, u-PA, FAK, pJNK, pERK, IKK, NF-κB, Bcl-2, Bcl-xL, Δψm, increased ROS, Ca$^{2+}$, cytochrome c, Bax, Bad, caspase 8/9/3, Apaf1, Fas, FADD, AIF, EndoG | 43,44 |
| 5-8F                 | 2.5, 5, 10, 20, 40, 80, or 100 µM | Decreased cell viability, motility, increased LDH, filopodia formation | Reduced Ererin phosphorylation (Thr$^{567}$) | 45,46 |
| CNE-1                | 2.5, 5, 10, 20 or 40 µg/mL | Decreased cell viability, cell migration, invasion, EMT | Reduced Twist, increased caspase 3 | 47 |

(continued)
| Cell line/IC<sub>50</sub>     | Berberine concentration                                                | Outcome                                                                                     | Mechanism                                                                                                                                                                                                 | References |
|------------------------------|------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| HONE1; HK1-EBV               | 12.5, 25, 75, 150, or 300 µM; 25, 50, 100, or 200 µM                  | Decreased cell proliferation, cell migration, invasion, stress fiber formation, increased apoptosis, G<sub>2</sub>/M arrest | Increased cdc2 (p-cdc2; Tyr15), PARP, caspase 3/9, decreased RhoGTPases, p-histone 3, EBNA1, STAT-3                                                                                                           | 48, 49     |
| KYSE-30                     | 1, 2, 4, 8, 16, 32, 64, 128 or 256 µM                                 | Decreased cell viability, cell migration                                                     | Decreased CCR7, CXCR4                                                                                                                                                                                  | 50         |
| KYSE-70; SKGT4               | 20, 40, 60, 80, and 100 µmol/L                                       | Decreased cell growth, increased apoptosis, G<sub>2</sub>/M arrest                           | Decreased Akt<sup>Ser47</sup>, mTOR<sup>Ser2448</sup>, p70S6K<sup>Thr389</sup>, increased AMPK<sup>Thr172</sup>                                                                                       | 51         |
| KYSe-450; TE-1; Eca109       | NR                                                                    | Decreased cell migration, invasion, EMT                                                     | NR                                                                                                                                                                                                     | 52         |
| FaDu                        | 12.5 or 25 µM                                                         | Increased cytotoxicity, nuclear condensation, apoptosis, decreased cell migration           | Increased Fasl, TRAIL, caspase 8/7/3, PARP, p53, Bad, Apaf-1, caspase-9, decreased Bcl-2, and Bcl-xl, VEGF, MMP-2/9, MAPK                                                                                    | 53         |
| SSC-15/ 235, SSC-4/242 µM   | 100, 150, 200, 250, or 300 µM                                        | Decreased cell viability, colony formation, increased autophagy, apoptosis                 | Conversation LC-3I to LC-3II, decreased SQSTM1 protein p62, miR-21, increased caspase 3, PARP, miR-155                                                                                                  | 54         |
| Gastrointestinal cancer      |                                                                        |                                              |                                                                                                                                                                                                         |            |
| SW620                       | 5, 10, 25, or 50 µM                                                   | Decreased cell viability, increased apoptosis                                              | Increased caspase 3/8, PARP, cytochrome c, ROS, JNK, p38 MAPK, phospho-c-Jun, Fasl, t-Bid, decreased Bid, c-IAP1, Bcl-2, Bcl-XL                                                                         | 55         |
| HCT-116 SW480               | 1, 10, or 50 µM                                                       | Decreased cell proliferation, increased apoptosis                                           | Increased NAG-1, ATF-3, caspase 3/7                                                                                                                                                                      | 56         |
| SNU5                        | 25, 50, 75, or 100 µM                                                 | Decreased cell viability, invasion                                                         | Increased ROS, decreased NF-κB, MMP-1/2/9                                                                                                                                                              | 57         |
| HCT118; SW480               | 1, 2, 5, 10, 20, 50, or 100 µM                                       | Decreased cell viability and cell migration                                                 | Increased ROS, AMPK, decreased, integrin β1, Src, FAK, p130Cas                                                                                                                                         | 58         |
| SW480                       | 0.5, 1, 2.5, 5, 10, 25, or 50 µM                                      | Decreased cell proliferation, increased apoptosis, G<sub>2</sub>/G<sub>1</sub> arrest        | Increased p21, cytochrome c, Bax, caspase 8/9/3, PARP, decreased VEGF, AIF, NF-κB, COX-2                                                                                                                                 | 59         |
| HCT116                      | 5, 10, 20, 40, or 80 µM                                               | Decreased cell proliferation, increased apoptosis, G<sub>2</sub>/G<sub>1</sub> arrest        | Decreased β-catenin                                                                                                                                                                                    | 60         |
| HCT-8                       | 0.03, 0.06, 0.12, 0.24, or 0.47 mmol/L                               | Decreased cell proliferation, S-arrest                                                     | Increased, LDH, alkaline phosphatase, acid phosphatase, TNF-α, Fasl, p53, prohibitin, Fas, Bax, caspase-3, decreased Bcl-2, procaspase-3, vimentin                                                                 | 61         |
| HCT116; KM12C               | 6.25, 12.5, 25, or 50 µM                                             | Decreased cell proliferation, colony formation, glucose uptake                           | Decreased GLUT1, LDH A, hexokinases 2 mRNA, HFI1, mTOR signaling                                                                                                                                     | 62         |
| HCT116                      | 1, 10, or 100 µM                                                      | Decreased cell viability, increased apoptosis                                              | Increased caspase-3, decreased miR-21, ITGβ4, PDCD4                                                                                                                                                   | 63         |
| DLD-1; Caco-2               | 6.25, 12.5, 25, or 50 µM                                             | Decreased cell proliferation, colony formation, S-phase fraction, G<sub>2</sub>/G<sub>1</sub> arrest | Decreased lipogenesis, ACC, ACL, FASN, β-catenin signaling, SREBP-1, SCAP                                                                                                                                | 64         |
| Cell line or type | Berberine concentration | Mechanism | Outcome | References |
|------------------|--------------------------|-----------|---------|------------|
| BGC-823; SGC7901 | 10, 25, 50, 75, or 100 µM | Decreased cell proliferation, increased apoptosis | BGC-823; SGC7901 | 65 |
| BGC-823; SGC7901 | 6.25, 1.5, 25, 50, 100, and 200 µM | Increased PARP, caspase-3, decreased Akt/mTOR/p70S6K | BGC-823; SGC7901 | 67 |
| SNU-1/30 µM; GES-1/120 µM | 3.125, 6.25, 12.5, 25, 50, or 100 µM | Decreased cell viability, increased apoptosis | SNU-1/30 µM; GES-1/120 µM | 68 |
| Caco-2/39.87; LOVO/23.27 µM | 10, 20, 40, 60, or 80 µM | Decreased cell viability, colony formation | Caco-2/39.87; LOVO/23.27 µM | 70 |
| LOVO/40.8 µM | 5, 25, 50, or 100 µM | Decreased cell viability, colony formation, G<sub>2</sub>/M arrest | LOVO/40.8 µM | 72 |
| Huh-7; HepG2; Hep3B; SNU-182 | 10, 20, 50, or 100 µM | Decreased cell proliferation, increased apoptosis, G<sub>2</sub>/M arrest | Huh-7; HepG2; Hep3B; SNU-182 | 74 |
| SKBR-3; BT-474; T47D; MDA-MB-231 | 5, 25, 50, or 100 µM | Decreased cell proliferation, increased apoptosis, G<sub>2</sub>/M arrest | SKBR-3; BT-474; T47D; MDA-MB-231 | 76 |
| MCF-7; MDA-MB-231 | 30, 60, or 120 µM; 50 µM | Decreased cell viability, colony formation, G<sub>0</sub>/G<sub>1</sub> arrest | MCF-7; MDA-MB-231 | 78 |
| MCF-7; MDA-MB-231 | 1.25, 2.5, 5, 10, 20, 40, 80, or 160 µM | Decreased cell growth, colony formation, G<sub>2</sub>/M arrest | MCF-7; MDA-MB-231 | 80–82 |
| Huh7; HepG2; Hep3B; SNU-182 | 5, 25, 50, or 100 µM | Decreased cell viability, increased apoptosis | Huh7; HepG2; Hep3B; SNU-182 | 83 |
| HepG2 | 1.5 µM | Decreased glucosidic receptors, α-lactalbumin | HepG2 | 85 |
| Cell line/Concentration | Berberine concentration (µM) | Mechanism | Outcome | References |
|-------------------------|-----------------------------|-----------|---------|------------|
| MCF-7                   | 10, 25, 50, 75, or 100 µM   | Decreased cell viability, colony formation, cell migration, invasion, increased apoptosis | MCF-7: Decreased cell survival, targeted berberine was more effective | 86 |
| MDA-MB-231              | 10, 20, 30, 40, or 50 µM    | Decreased cell viability, colony formation, cell migration, invasion, increased apoptosis | MDA-MB-231: Decreased cell survival, targeted berberine was more effective | 87 |
| BT549                   | 5, 10, 20, 40, and 80 µM    | Decreased cell viability, colony formation, cell migration, increased apoptosis | BT549: Decreased cell viability, colony formation, cell migration, increased apoptosis | 88 |
| Hs578                   | 25 or 50 µM                | Decreased cell viability, increased apoptosis | Hs578: Decreased cell viability, increased apoptosis | 89 |
| MCF-7                   | 3, 6, or 12 µM, 6.25, 12.5, 25, or 50 µM | Decreased cell proliferation, G1 arrest (MDA-MB-468/BT-549 cells) | MCF-7: Decreased cell proliferation, S+G2/M arrest (MDA-MB-468/BT-549 cells) | 90 |
| MCF-7                   | 1, 10 or 100 µM            | Decreased cell proliferation, G1 arrest (MDA-MB-468/BT-549 cells) | MCF-7: Decreased cell proliferation, S+G2/M arrest (MDA-MB-468/BT-549 cells) | 91 |
| MCF-7                   | 2, 5, or 10 µM             | Decreased cell proliferation, G1 arrest (MDA-MB-468/BT-549 cells) | MCF-7: Decreased cell proliferation, G1 arrest (MDA-MB-468/BT-549 cells) | 92 |
| MCF-7                   | 0.1, 1, 5, 10, 50, 100, or 150 µM | Increased DNA fragmentation | MCF-7: Increased DNA fragmentation | 93 |
| Hela                    | 125, 250, 500, or 1000 µM   | Decreased cell viability, growth, increased apoptosis | Hela: Increased cell viability, growth, increased apoptosis | 94 |
| CaSki                   | 50, 100, or 150 µM         | Decreased cell viability, growth, increased apoptosis | CaSki: Decreased cell viability, growth, increased apoptosis | 95 |
| HeLa                    | 1, 250 µg/mL               | Increased DNA fragmentation | HeLa: Increased DNA fragmentation | 96 |
| Hela/HeLa               | 150, 275, 500, 225, 500, 275, or 300 µM | Increased DNA fragmentation | Hela/HeLa: Increased DNA fragmentation | 97 |
| HeLa/283                | 50, 100, or 150 µM         | Increased DNA fragmentation | HeLa/283: Increased DNA fragmentation | 98 |

Table 1. (continued)
### Table 1. (continued)

| Cell line/IC₅₀ | Berberine concentration | Outcome | Mechanism | References |
|----------------|-------------------------|---------|-----------|------------|
| HeLa           | 1, 2, 4, 6, or 8 µg/mL; 10, 50, or 100 µM | Decreased cell viability, increased apoptosis, DNA fragmentation, G₂/M arrest | Complexed with DNA, decreased nucleophosmin/B23 mRNA, telomerase, NAT, 2-aminofluorene (AF)-DNA adduct, N-cadherin, increased E-cadherin | 105-108 |
| HL-60; WEHI-3 (mouse) | 5, 15, 30, or 60 µM | Increased cytotoxicity, apoptosis, G₂/G₁, G₂/M arrest | Increased Ca²⁺, caspase-3, Bax, cytochrome c, Wee1, 14-3-3σ, decreased Δψm, Bcl-2, Cdc25c, CDK1, cyclin B1, Src | 109,110 |
| HL-60 | 2.5, 5, 10, 20, 40, 80, or 100 µM; 20, 40, 60, 80, or 100 µM | Decreased cell viability, migration, increased apoptosis, chromatin condensation, DNA fragmentation | No change in CXCR-4, increased PARP, caspase 3/8, ERK, p38 | 111,112 |
| EU4 | 1, 10, 50, or 100 µM | Increased cytotoxicity, apoptosis, | Increased caspase 3/9, PARP, Bax, mIr-24-3p, PIM-2, decreased XIAP, MDM2 | 113,114 |
| EU6 | 12.5, 25, 50, or 100 µM | Decreased cell viability, increased autophagy | Decreased AKT/mTORC1, p-S6, pAKT | 115 |
| MM.1S/15-25 µM; RPMI-8266 | 25, 50, 75, and 100 µM | Decreased cell viability, colony formation | Increased p16^{INK4A}, p73, UHRF1 degradation | 116 |

### Prostate cancer

| Cell line | Berberine concentration | Outcome | Mechanism | References |
|-----------|-------------------------|---------|-----------|------------|
| DU145; PC-3; LNCaP | 10-100 µM; 25, 50, 75, or 100 µM (PC-3) | Decreased cell proliferation, increased cell death, DNA fragmentation, apoptosis, G₂/G₁ arrest | Decreased cyclins D1/2E, CDK 2/4/6, Δψm, increased p21^{CIP1}, p27^{KIP1} Bax, caspase 9/3, PARP, ROS, cytochrome c, Smac/DIABLO | 117-118 |
| PC-3; LNCaP | 5, 10, 20, 50, or 100 µM | Decreased cell growth, increased apoptosis, G₂ arrest | Increased Bax, caspase 3 | 119 |
| LnCaP; PC-3 | 20, 100, or 200 µM | Decreased cell growth, increased apoptosis, G₂ arrest | Decreased prostate-specific antigen, EGFR | 120 |
| LNCaP; LAPC-4; 22Rv1; C4-2B, PC-3 | 1.56, 3.125, 6.25, 12.5, 25, 50, or 100 µM | Decreased cell proliferation, increased apoptosis | Decreased androgen receptor | 121 |
| RM-1 (mouse); PC-3 | 5, 10, 20, or 50 µM | Decreased cell proliferation, increased apoptosis, G₂/G₁, G₂/M arrest | Increased DNA DSBs, p53, p21, ATM/Chk1 | 122,123 |
| Cell line/IC_{50} | Berberine concentration | Outcome | Mechanism | References |
|------------------|-------------------------|---------|-----------|------------|
| LNCaP; PC-82 | 1, 5, 25, 50, or 100 µM | Decreased cell viability, increased apoptosis, necrosis | Increased cyclophilin-D, p53 translocation to mitochondria | 124 |
| PC-3; DU 145; LNCaP | 10, 25, 50, 75 µM | Decreased cell proliferation, motility, migration, EMT | Decreased vimentin, PDGFRβ, COL1A2, BMP7, TGF-β, NODAL, WNT1, Snail | 125 |
| 22Rv1 | 12.5, 25, or 50 µM | Decreased cell proliferation, increased apoptosis | Decreased C3 enzyme | 126,127 |
| Ovarian cancer | OVCAR-3/10 µM; SKOV-3/100 µM | 1, 10, or 100 µM | Decreased cell proliferation, G_{2/M}, S-arrest | Increased p27 | 128 |
| FTE187; A2780; HEY; HO8910 | 5, 10, or 20 µM | Decreased proliferation, colony formation, increased apoptosis | Increased ROS, PARP, ATM, p53, DNA DSBs, decreased RAD51, homologous recombination DNA repair | 129 |
| SKOV3/9.2 µM | 5, 10, 30, 50 or 100 µM | Decreased cell proliferation, cell migration, invasion | Decreased hERG1 | 130 |
| SKOV-3/50 µM; TOV-21G/25 µM; MDAH-2774/52 µM | 12.5, 25, 50 and 100 µM | Decreased cell viability, colony formation, cell migration, invasion, increased cytotoxicity, | Decreased EGFR, ErB2, cyclin D1, MMP 2/9, VEGF, PI3K, Akt | 131 |
| SKOV3/78.52 µM; 3AO/125.8 µM | 2.5, 5, 10, 20, 40, 80, 160, or 320 µM | Decreased cell proliferation, cell migration, invasion | Increased miR-145, TET3, HK2, decreased MMP16, Warburg effect, | 132,133 |
| Osteosarcoma | U2OS; Saos-2; HOS | 1, 5, 10, 20, or 50 µg/mL | Decreased cell proliferation, increased apoptosis, G_{1} arrest | Increased p53, p21, p27, Bax, PUMA, FAS, DNA DSBs, decreased cyclin E/D1 | 134 |
| U2OS | 12.5, 25, or 50 µg/mL | Decreased cell proliferation, colony formation, increased apoptosis | Decreased PI3k, AKT, Bcl2, procaspase-3, increased PARP, Bax | 135 |
| Saos-2; MG-63 | 10, 20, 40, 60, 80, or 100 µg/mL | Decreased cell proliferation, increased apoptosis | Decreased caspase 1, IL-1β | 136 |
| MG-63 | 20, 40, 60, or 80 µM; 5, 10, 20, 40, or 80 µM | Increased cytotoxicity, apoptosis, DNA fragmentation, decreased colony formation, EMT | Increased DNA DSBs, decreased MMP-2, N-cadherin, vimentin, fibronectin, β-catenin, snail, EZH2 | 137,138 |
| Lung cancer | A549 | 2.5, 5.0, 10, 20, 40, 80, or 100 µM | Decreased cell proliferation, cell migration, invasion, G_{1} arrest | Increased TIMP-2, Akt, CREB, MAPK, decreased MMP-2, uPA, NF-κB, cFos, cJun, cyclin B1 | 139,140 |
| A549; H1299 | 25, 50, 75, or 100 µM | Decreased cell proliferation, increased DNA fragmentation, apoptosis | Increased Bax, Bak, caspase 3, decreased Δψm, Bcl-2, Bcl-xL | 141 |
| H460/5 µM | 0.1, 1, 5, or 10 µM | Decreased cell growth, G_{0}/G_{1} arrest | NR | 142 |
| Cell line/IC₅₀ | Berberine concentration | Outcome | Mechanism | References |
|---------------|-------------------------|---------|-----------|------------|
| A549          | 20, 40, 80, or 160 µM; 6.25, 12.5, 25, 50, or 100 µM | Decreased cell viability, cell migration, invasion, EMT, increased apoptosis, G₁/G₀ arrest | Increased E-cadherin, p21, ROS, p21⁰⁵⁺⁶¹, IL6, IL1β, TNF-α, p-NF-κB, mTOR, decreased vimentin, Snail-1, Slug, cyclin A₁/2/B1, NF-κB | 143-145 |
| A549          | 20, 40, 80, 100, 120, 140, 160, 180, or 200 µmol/mL | Decreased cell viability, increased nuclear fragmentation, apoptosis, G₁/G₀ arrest | Increased ROS, p21, p53, Bax, cytochrome c, caspase 8/9/3, decreased Δψm, Bcl-xl, Bcl-2, TNF-α, COX-2, MMP-2, & MMP-9, HDAC 1/2/4 | 147 |
| A549          | 30, 60, 90, 150, or 200 µM | Decreased cell proliferation, increased apoptosis | Increased Bax, decreased MOMP-2, Janus kinase-2, VEGF, NF-κB, N-P1 | 148 |
| A549; PC9     | 20, 40, 60, 80, 100, 120, 140, or 160 µM | Decreased cell proliferation, colony formation, increased apoptosis | Increased Bax, TF, JNK, p38MAPK, decreased Bcl2, miR-19a | 149 |
| NCI-H460/30.3 µM; A549/44.5 µM; NCI-H1299/43.8 µM | 10, 20, 40 or 80 µM | Decreased cell proliferation, colony formation, increased apoptosis | Increased DNA DSBs, decreased TOP2B, SIN3A | 150 |
| Pancreatic cancer | BxPC-3/6.28 µM; HPDEE6E7c7 | 10, 50, 100, 150, or 200 µM | Decreased cell survival, increased apoptosis, DNA damage | Increased caspase 3/7, AIF, 234 genes, decreased 33 genes related to BRCA1-mediated DNA damage response, G₁/G₀, G₂/M cell cycle checkpoint regulation, p53 signaling | 151 |
| PANC-1/15 µM; MiaPaCa-2/10 µM | NR; 1, 5, 7, 10, or 15 µM | Decreased side population of cells, cell proliferation, increased apoptosis, G₁ arrest | Decreased SOX2, POU5F1, NANOG, increased ROS | 152,153 |
| PANC-1, MiaPaCa-2 | 0.15, 0.3, 1.5, 3, or 6 µM | Decreased cell proliferation, G₁ arrest | Decreased DNA synthesis, Δψm, ATP, mTORC1, ERK, increased AMPK, acetyl-CoA carboxylase | 154 |
| PANC-1        | 1, 5, 7.5, 10, 15, or 30 µM | Decreased cell proliferation, migration, increased apoptosis | Decreased TNF-α, K-ras, 3726 genes, increased 3726 genes, CDKN2A, glycolysis | 155 |
| Renal cancer  | ACHN | 10, or 20 µmol/L | Decreased cell proliferation | Decreased c-Fos | 156 |
| G401          | 5, 10, 20, or 50 µM | Decreased cell proliferation | Increased p21, p27, AMPK, T-ACC, mTOR, S6 kinase, WTX, decreased cyclin E | 157 |
| Bladder cancer | T24 | 0.8, 8, 80, 800, or 1,600 µM | NR | Decreased NAT | 158 |
| BIU-87; T24 | 1, 5, 10, 25, 50, 75, or 100 µM | Decreased cell viability, increased apoptosis, G₁/G₀ arrest | Increased caspase-3/9, H-Ras, c-fos | 159 |
| T24          | 10, 25, or 50 µg/mL | Decreased cell migration, invasion | Decreased heparanase | 160 |
| Thyroid cancer | 8S05C/10 µM; TPC1/10 µM | 1, 10, or 100 µM | Decreased cell growth, G₁/G₀ arrest | Increased p-27 | 161 |

(continued)
**Table 1. (continued)**

| Cell line/C50 | Berberine concentration | Outcome | Mechanism | References |
|---------------|-------------------------|---------|-----------|------------|
| FTC-133; B305C | 10, 25, 50, or 100 µM | Decreased cell viability, increased apoptosis, DNA fragmentation | Increased caspase 3, p53, p27 | 162 |
| TT/1 µg/mL | 0.4, 0.8, 1.6, 3.2 and 6.4 µg/mL | Decreased cell viability, S/G2 phase fraction, increased apoptosis, G1 arrest | Increased caspase 3, decreased RET, Akt, Bcl2, Bcl2, E2F1, cyclin E | 163 |
| C643; OCUT1; TPC1 | 10, 20, 40, 80, or 160 µM | Decreased cell proliferation, increased apoptosis, arrested G0/G1 phase | Decreased Δψm, cyclin E1, CDK2, vimentin, p-AKT1, p-AKT1, p-ERK, p-JNK, P38K, p-Akt, Akt, Nrf2, increased caspase 3, Bax, p-21, p-ERK, p-P38, p-JNK | 164 |
| K1 | 10, 40, or 80 µmol/L | Decreased cell proliferation | Decreased P38K, p-Akt/Akt, Nrf2 | 165 |

NR, not reported; ACC, acetyl-CoA carboxylase; ACL, ATP citrate lyase; AIF, apoptosis-inducing factor; AMPK, AMPK-activated protein kinase; AP, activator protein; Apaf, apoptotic protease activating factor; ATF, activating transcription factor; ATM, ataxia telangiectasia mutated; ATP, adenosine triphosphate; Bad, Bcl-2 antagonist/killer; Bax, Bcl2-associated X apoptosis regulator; Bd, B-cell lymphoma; BID, BH3 interacting domain death agonist; BMP, bone morphogenetic protein; C/EBP, CCAAT/enhancer-binding protein; CCR, C-C chemokine receptor type; Cdc, cell division cycle; CDK, cyclin-dependent kinase; CDKIs, cyclin-dependent kinase inhibitors; COX, cyclooxygenase; CXR, C-X-C motif chemokine receptor; DDB1, DNA damage inducible gene; DSBs, double-strand breaks; EBNA1, Epstein-Barr nuclear antigen 1; EGFR, epidermal growth factor receptor; EMT, epithelial-to-mesenchymal transition; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; ETV, erythroleukemia transcription initiation factor; FAOD, FAS-associated death domain; FAK, focal adhesion kinase; FASN, fatty acid synthase; FoxO3a, forkhead box O3a; GADD, growth arrest and DNA damage-inducible genes; GIP, glucose-regulated protein; GH, glutathione; HDAC, histone deacetylase; GSK, glycogen synthase kinase; hERG, human ether-a-go-go-related gene; HIF, hypoxia-inducible factor; HICBP, immunoglobulin heavy chain binding protein; IL, interleukin; JNK, C-Jun N-terminal kinase; LC3, microtubule associated proteins 1A/1B light chain 3B; LDH, lactate dehydrogenase; M6K/MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; MRP, mitochondrial ribosomal protein; mTOR, mechanistic target of rapamycin; NADPH, nicotinamide adenine dinucleotide phosphate hydrogen; NAG, nonsteroidal anti-inflammatory drug activated gene; NAM, N-acetylamino acid; NAMPT, N-acetyltransferase; Nrf2, nuclear factor E2-related factor; O6, nuclear factor kappa B; p-AKT1, p-AKT1, p-ERK, p-JNK, P38K, p-Akt, Akt, Nrf2, increased caspase 3, Bax, p-21, p-ERK, p-P38, p-JNK | 165 |

NF-κB, nuclear factor kappa B; Notch, neural progenitor notch homolog protein; PARP, poly(ADP-ribose) polymerase; PCNA, proliferating cell nuclear antigen; PTF1, pentatricopeptide repeat domain; PTTG, pituitary tumor transforming gene; RAF, rapidly accelerated fibrosarcoma; Ras, retrovirus-associated DNA sequences; ROS, reactive oxygen species; SCAP, SREBP cleavage activating protein; Sipk, S-phase kinase-associated protein; SQSTM1, sequestosome-1; SREBP, sterol regulatory element-binding protein; STAT, signal transducer and activator of transcription; TCF, T-cell factor; TGF, transforming growth factor; TIF, translation initiation factor; TRAIL, tumor necrosis factor (TNF)-related apoptosis-inducing ligand; TUMF, Tu transcription elongation factor; ULK1, Unc-51 like autophagy activating kinase; VASP, vasodilator-stimulated phosphoprotein; VEGF, vascular endothelial growth factor receptor; Wnt, Wingless-type MMTV integration; WTX, Wilms tumor gene on X chromosome; XIAP, X-linked inhibitor of apoptosis protein; Δψm, mitochondrial membrane potential.
Berberine treatment reduced the migration and invasion of U87 and U251 cells and induced apoptosis by upregulating Bax, cytochrome c release, caspase 3 activation, and reducing Bcl-2 protein expression. Berberine increased oxidative phosphorylation and reduced markers of glycolysis: adenosine triphosphate (ATP), L-lactate, and lactate dehydrogenase (LDH; Table 1). Berberine increased concentration-dependent autophagy by upregulating microtubule-associated protein 1A/1B-light chain 3 (LC3)-II and downregulating sequestosome-1 (SQSTM1)/p62 proteins. Berberine elevated AMP-activated protein kinase (AMPK), Beclin-1, and phosphorylated Unc-51-like autophagy activating kinase 1 (ULK-1), a downstream target for mechanistic target of rapamycin (mTOR) depending on the concentration in U87 and U251 cells. Berberine (50 or 100 µM) induced the death of U251 and U87 cells and inhibited cell migration, production of interleukin (IL)18 and IL1β, and epithelial-to-mesenchymal transition (EMT) by reducing the activation of ERK1/2 depending on its concentration (Table 1).

Exposure of Na2 (mouse neuroblastoma) and IMR-32 (human neuroblastoma) cells to 10 and 20 µg/mL berberine reduced cell proliferation, increased cell differentiation, and alleviated changes in stemness-related markers of cancer including CD133, β-catenin, n-myc, sex determining region Y-box 2 (Sox2), Notch2, and neuroepithelial stem cell protein (Nestin). Berberine arrested cells in the G0/G1 phase of the cell cycle, inhibited CDK-2/4 and cyclin D1/E, enhanced the Bax/Bcl-2 ratio, and increased p27 and p53 expression (Table 1). Berberine exhibited antimigratory potential by inducing neural cell adhesion molecule (NCAM), attenuating its polysialylation, and downregulating matrix metalloproteinase (MMP)-2/9. Berberine treatment enhanced the epithelial marker its polysialylation, and downregulating matrix metalloproteinase by inducing neural cell adhesion molecule (NCAM), attenuating expression (Table 1). Berberine exhibited antimigratory potential D1/E, enhanced the Bax/Bcl-2 ratio, and increased p27 and p53 levels of ERK1/2 in a concentration-dependent manner. An oncosis-like death was triggered, characterized by membrane blebbing, and intracellular ATP decline. Berberine increased ROS formation, Ca2+ release in the cytosol, and reduced markers of glycolysis: adenosine triphosphate (ATP), lactate, and lactate dehydrogenase (LDH; Table 1).

The exposure of esophageal carcinoma cells KYSE-30 to 1, 2, 10, 20, 40, 80, and 100 µM berberine caused a time- and concentration-dependent manner and downregulation of Epstein–Barr nuclear antigen 1 (EBNA1) and signal transducer and activator of transcription 3 (STAT3) at the mRNA level (Table 1). The exposure of esophageal carcinoma cells KYSE-30 to 1, 2, 10, 20, 40, 80, and 100 µM berberine reduced cell viability and increased LDH release, especially after 40 µM. Berberine inhibited the invasion and motility of 5-8F cells in a time and concentration-dependent manner by inhibiting filopodia formation and downregulating Ezrin phosphorylation at Thr392 (Table 1). NPC CNE-1 cells treated with 2.5, 5, 10, 20, 40 or 40 µg/mL berberine hydrochloride had reduced cell viability in a concentration- and time-dependent manner and diminished cell migration, invasion, and EMT through decreased Twist expression. Berberine triggered apoptosis and activation of caspase 3 in CNE-1 cells (Table 1). NPC HONE1 cells treated with 12.5, 25, 75, 150, and 300 µM berberine reduced cell proliferation in a concentration-dependent manner and its distribution in the cells was also dependent on the concentration of berberine treated cells. Berberine also reduced cell migration, invasion, RhoGTPases, formation of stress fibers (at low concentrations), and arrested cells in the G2/M phase of the cell cycle by activating Cdc2 (p-Cdc2; Tyr15) in addition to reducing the expression of p-histone 3. Berberine triggered apoptosis and the activation of PARP and caspase 3/9. HONE1 and HK1-EBV cells treated with 25, 50, and 100 µM berberine exhibited a reduction in cell viability in a concentration-dependent manner and downregulation of Epstein–Barr nuclear antigen 1 (EBNA1) and signal transducer and activator of transcription 3 (STAT3) at the mRNA level (Table 1). The expression of esophageal carcinoma cells KYSE-30 to 1, 2,
but not MMP-7, which remained unaltered at the mRNA level (Table 1).57

Berberine treatment (1, 2, 5, 10, 20, 40, or 100 µM) reduced cell viability and cell migration and increased ROS generation, activated AMPK, and significantly decreased integrin β1 levels, phosphorylation of Src, FAK, and p130Cas in SW480 and HCT116 cells (Table 1).58 Exposure of SW480 cells to 0.5, 1, 2.5, 5, 10, 25, and 50 µM berberine decreased cell proliferation depending on the concentration and length of treatment time, and did not induce cytotoxicity in normal CCD-CoN121 colon cells up to 200 µM. Berberine arrested cells in the G0/G1 phase of the cell cycle, depleted Δψm, and decreased the expression of p21 (CDK4), cytochrome c, and Bax/Bcl2. Berberine activated caspase 9/3, AIF, and cleaved PARP, and reduced VEGF, NF-κB, and COX-2 expression; however, survivin and TRAIL expression remained unaffected (Table 1).59

HCT116 cells treated with berberine (5, 10, 20, 40, and 80 µM) showed reduced proliferation and elevated apoptosis in a concentration-dependent fashion, cells were arrested in the G1 phase of the cell cycle, and mRNA expression of β-catenin was inhibited in both the nucleus and cytoplasm (Table 1).60 Exposure of HCT-8 cells to 0.03, 0.06, 0.12, 0.24, or 0.47 mM berberine for 12, 24, 48, and 72 h resulted in reduced cell proliferation in a concentration and time-dependent manner and cells were arrested in the S-phase of the cell cycle. Berberine increased tumor necrosis factor alpha (TNF-α), alkaline phosphatase, acid phosphatase, the LDH levels, the expression of Fasl, p53, and prohibitin (PHB), and mRNA levels of Fas, FasL, and Bax, as well as the activation of caspase-3. Berberine reduced the expression of Bcl-2, procaspase-3, and vimentin (Table 1).61 Treatment of HCT116 and KM12C cells with 6.25, 12.5, 25, and 50 µM berberine reduced cell proliferation and colony formation in a concentration-dependent manner by inhibiting glucose uptake as indicated by the mRNA suppression of glucose transporter 1 (GLUT1), lactate dehydrogenase A, and hexokinases 2 (HK2), in addition to the expression of hypoxia inducible factor 1 (HIF1) protein and mTOR signaling; however, analysis by reverse transcription-polymerase chain reaction (RT-PCR) did not show any change in HIF1 mRNA (Table 1).62 Similarly, 1, 10, and 100 µM berberine depleted cell viability, increased levels of apoptosis, activated caspase-3, integrin β4 (ITGβ4), and programmed cell death 4 (PDCD4) protein expression, and inhibited mir-21 mRNA expression in HCT116 cells (Table 1).63

Berberine (6.25, 12.5, 25, and 50 µM) concentration-dependently reduced cell proliferation and colony formation and arrested DLD-1 and Caco-2 cells in the G0/G1 phase of the cell cycle and also depleted the S-phase fraction. Berberine also decreased glucose-induced lipogenesis in these cells and inhibited the mRNA expression of acetyl-CoA carboxylase (ACC), ATP citrate lyase (ACL), and fatty acid synthase (FASN), and decreased sterol regulatory element-binding protein-1 (SREBP-1) activation, SREBP cleavage-activating protein (SCAP) expression, and β-catenin signaling (Table 1).64

Exposure of BGC-823 and SGC7901 cells to 10, 25, 50, 75, and 100 µM berberine slowed cell proliferation in a time- and concentration-dependent manner, and also elevated apoptosis, expression of PARP, and caspase-3 while reducing Δψm. The BCG-823 cells were more sensitive to berberine than SGC7901 cells. Berberine downregulated the Akt/mTOR/p70S6/S6 pathway in BGC-823 cells indicating that the Akt-related mitochondrial pathway may be involved in berberine-induced apoptosis (Table 1).65 Exposure of BGC-823 cells to 14, 21, 32, 48, 72, and 108 µM berberine for 6, 12, 24, 36, and 48 h attenuated cell proliferation depending on the length of treatment time and concentration with an IC50 value

4, 8, 16, 32, 64, 128, or 256 µM berberine decreased cell viability and cell migration in a time- and concentration-dependent way by significantly reducing the expression of chemokine receptor 7 (CCR7) and C-X-C chemokine receptor type 4 (CXCR4; Table 1).56 Likewise, KYSE-70 and SKGT4 cells treated with 20, 40, 60, 80, and 100 µM berberine suppressed cell growth in a concentration- and time-dependent manner, increased apoptosis, and arrested cells in the G2/M phase of the cell cycle. Berberine treatment repressed Akt (Ser473), mTOR (Ser2448) and p70S6K (Thr389) phosphorylation but increased phosphorylation of AMPK at Thr172 (Table 1).57 Berberine suppressed the microRNA-212-induced cell migration, invasion, and EMT in KYSe-450, TE-1, and Eca109 cells (Table 1).58

Exposure of head and neck squamous cell carcinoma (HNSCC) FaDu cells to 12.5 or 25 µM berberine induced cytotoxicity, whereas the viability of primary human normal oral keratinocytes was unaffected. Berberine elevated nuclear condensation, apoptosis, Fasl, and TNF-related apoptosis-inducing ligand (TRAIL), activation of caspase 8/7/3, and PARP in FaDu cells. Berberine triggered the mitochondria-dependent apoptotic signaling pathway by elevating p53 and proapoptotic factors including Bad, Bax, Apaf-1, caspase-9, and downregulating Bcl-2 and Bcl-xL. Berberine inhibited FaDu cell migration by downmodulating vascular endothelial growth factor (VEGF), MMP-2/9, and suppressing the MAPK pathway (Table 1).59 HNSC SSC-15 and SSC-4 cells treated with 200, 250 or 300 µM berberine had significantly reduced cell viability depending on the concentration, with IC50 values of 235 and 242 µM for SSC-4 and SSC-15 cells, respectively. SSC-15 cells exposed to 100, 150, 200, and 250 µM berberine had significantly reduced clonogenic potential that was concentration-dependent, and autophagy was stimulated by the conversation of microtubule-associated protein 1 light chain 3β-II (LC3-II), a distinct hallmark of autophagosome maturation. Berberine drastically reduced SQSTM1/p62 expression and triggered apoptosis by activating caspase 3 and PARP1 cleavage at higher concentrations. Berberine upregulated tumor suppressor microRNA (miRNA)-155 and downregulated oncogenic miR-21 in SSC-15 cells (Table 1).60

Gastrointestinal cancer

Berberine at 5, 10, 25, and 50 µM reduced cell viability and increased apoptosis in a concentration-dependent manner in SW620 cells, and 50 µM berberine activated caspase 3/8, PARP cleavage, and increased cytochrome c release with a subsequent decline in BH3 interacting domain death agonist (Bid), and antiapoptotic factors cellular inhibitor of apoptosis 1 (c-IAP1), Bcl-2, and Bcl-xL expression. Berberine increased ROS generation, phosphorylation of JNK and p38 MAPK, and increased levels of phospho-c-Jun, Fasl, and t-Bid levels due to JNK and p38 MAPK signaling (Table 1).61 Treatment of HCT-116 and SW480 cells with 1, 10, and 50 µM berberine for 1, 2, and 4 days reduced cell proliferation concentration-dependently and SW480 cells responded more quickly compared to HCT-116 cells. Berberine elevated the expression of nested antisense gene 1 (NAG-1) protein in HCT and CaCo-2 cells, and activating transcription factor 3 (ATF-3) in HCT-116 and SW480 cells depending on p53 activation. Berberine increased apoptosis and caspase 3/7 activity in HCT-116 cells due to activation of NAG-1 and ATF-3 genes (Table 1).62 SNU5 cells treated with 25, 50, 75, and 100 µM berberine showed a reduction in cell viability and cell invasion in a concentration-dependent manner. Berberine treatment also enhanced ROS formation up to 6 h and downregulated the protein expression of NF-κB and MPP-1/2/9, DOI: 10.14218/JERP.2021.00005 | Volume 00 Issue 00, Month Year
of 24.16 ± 1.02 µM (48 h). Berberine (25 µM) induced autophagy, increased the number of autolysosomes, increased the expression of Beclin-1, LC3-II, and p-ULK1, and attenuated the phosphorylation of Akt, ERK, JNK, and p38 depending on the treatment duration. (Table 1).66

Treatment of SNU-1 neoplastic and GES-1 non-cancerous cells with 6.25, 12.5, 25, 50, 100, and 200 µM berberine induced cytotoxicity and inhibited cell migration and invasion in a concentration-dependent manner, and its effect was more pronounced in SNU-1 cells (IC$_{50}$ of 30 µM) than in GES-1 cells (IC$_{50}$ of 120 µM). Berberine triggered apoptosis, activation of caspase-3/8/9, and repressed the activation of NF-$\kappa$B depending on its concentration in SNU-1 cells (Table 1).67 Exposure of Caco-2 and LoVo cells to 10, 20, 40, 60, and 80 µM berberine concentration-dependently reduced cell viability and colony formation with IC$_{50}$ values of 39.87 and 23.27 µM for Caco-2 and LoVo cells, respectively. Berberine increased levels of cleaved-PARP and activated caspase 3 but did not decrease cyclin D1 expression. Proteomic profiling revealed that 503 and 277 proteins were differentially expressed (DEPs) in Caco-2 and LoVo cells, out of 8051 identified proteins, and there was an overlap of 83 downregulated DEPs. Analysis of citrate synthase (CS), Tu translation elongation factor (TUFM), pentatricopeptide repeat domain 3 (PTCD3), and mitochondrial ribosomal protein L48 (MRPL 48) showed a decline, whereas CS protein expression was greater in Caco-2 and LoVo cells than in normal specimens (Table 1).68 Treatment of LoVo cells with 1.25, 2.5, 5, 10, 20, 40, 80, or 160 µM berberine for 24, 48, and 72 attenuated cell growth and colony formation in a concentration-dependent manner with an IC$_{50}$ of 40.8 ± 4.1 µM. Berberine arrested the cells in the G_{0}/G_{1} phase of the cell cycle and inhibited the protein expression of cyclin B1, Cdc2, and Cdc25c in addition to DNA and protein synthesis (Table 1).69

Liver cancer

Human hepatocellular carcinoma (HCC) HepG2 cells treated with 1–50 µM or 5, 25, 50, 100, and 200 µM or 10, 50 and 100 µM berberine for 12, 24, and 48 h berberine have shown a concentration-dependent decline in cell growth through the stimulation of apoptosis, and cells were arrested in the S- and G_{2}/M phases of the cell cycle. There was also reduced glucocorticoid receptor expression, α-fetoprotein secretion, and decreased levels of specificity protein 1 (SP1), cyclin D1, Bcl-2 and NF-$\kappa$B (Table 1).70–72 Berberine (3.125, 6.25, 12.5, 25, 50, and 100 µM for 24 or 48 h) depleted cell viability depending on the length of treatment time and concentration in HepG2, SMMC-7721, and Bel-7402 HCC cells when compared to normal hepatocytes (HL-7702 cells). Berberine increased apoptosis, the ratio of Bax/Bcl-2, activation of caspase 9/3, phosphorylation of AMPK and Akt and cytochrome c released from the mitochondria (Table 1).73 Berberine stimulated the expression of miR-23a in MHCC97L and PLC/PRF/5 HCC cells depending on its concentration and it transcriptionally activated p21 and GADD45 leading to p53 activation (Table 1).74 Human HepG2 and Bel-7404 and H22 (murine) hepatoma cells and normal hepatic embryo HL-7702 cells treated with 0, 12.5, 25, 50, or 100 µM berberine for 24 h showed a concentration-dependent decline in cell proliferation, and increased apoptosis and activation of caspase 9/3 in HepG2 cells. Berberine suppressed cytosolic phospholipase A$_2$ (cPLA2) and COX-2 expression in H22 and HepG2 cells (Table 1).75

The expression of HepG2, HepB3, and SNU-182 cells to 10, 20, 50, and 100 µM berberine concentration-dependently arrested cell proliferation and upregulated the expression of Kriippel-like factor 6 (KLF6), ATF3, and p21 at 100 µM in HepG2 cells, whereas no such effect was detected in HepB3 or SNU-182 cells. Berberine decreased the expression of the E2F transcription factor 1 (E2F1) and pituitary tumor transforming gene 1 (PTTG1) (Table 1).76 Berberine (30, 60, and 120 µM for 12–72 h) treatment of Huh-7 and HepG2 cells reduced cell viability and clonogenicity in a concentration-dependently manner, and Huh-7 cells were more sensitive than HepG2 cells. Berberine arrested Huh-7 and HepG2 cells in the G_{0}/G_{1} phase of the cell cycle depending on the concentration and deactivated the Akt pathway, inhibited the S-phase kinase-associated protein 2 (Skp2) expression, and elevated the expression and translocation of Forkhead box O3a (FoxO3a) into the nucleus, which promoted the transcription of the cyclin-dependent kinase inhibitors (CDKIs) p21$^{Cip1}$ and p27$^{Kip1}$ (Table 1).77 Likewise, 50 µM berberine induced a concentration- and time-dependent reduction in β-catenin (independent of APMK activation) and suppressed p-p70S6K$_{Thr389}$ and p4EBP1$_{Thr37/46}$ levels in addition to the mTORC1 axis in Huh7 and Hep3B cells. Berberine increased pAKT$_{Ser473}$ and pGSK3$\beta$Ser9 (downstream) levels due to activation of mTORC2.78 Treatment of human cholangiocarcinoma cells KKU-213 and KLU-214 with 2, 4, 6, 8, 20, and 20 µM berberine inhibited cell growth depending on the concentration, arrested cells in the G_{1} phase of the cell cycle, and suppressed the activation of STAT-3, NF-$\kappa$B, and phosphorylation of ERK-1/2 (Table 1).79

Breast cancer

Exposure of MCF-7 and MDA-MB-231 cells to 0.001, 0.01, 0.1, 1, 10, 20, 50, and 100 or 500 µM berberine decreased cell proliferation in a concentration- and time-dependent fashion and elevated apoptosis. Berberine arrested cells in the G_{0}/G_{1} phase of the cell cycle, attenuated cell migration and invasion, and interacted with vasodilator-stimulated phosphoprotein (VASP) to inhibit actin polymerization (Table 1).80–82 Similarly, 0–100 µg/mL berberine reduced cell proliferation in a concentration-dependent manner (IC$_{50}$ 36.91 µg/mL) and increased ROS generation in MCF-7 cells. MCF-7 cells treated with berberine (36.91 µg/mL) expressed 1800 well-defined proteins, out of which 96 proteins were DEPs as indicated by matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) analysis. In MCF-7 cells, berberine downregulated the proteins involved in proteolysis, protein folding, cell signaling, redox regulation, electron transport, and metabolism, and upregulated the proteins involved in protein trafficking including Hsp27 (Table 1).83 Exposure of SKBR-3 (human epidermal growth factor receptor 2 [HER2$^{+}$], BT-474 (HER2$^{+}$), T47D (HER2$^{+}$), and MDA-MB-231 (HER2$^{+}$) breast cancer cells to 20, 40, 60, 80, and 100 µM or 10, 25, 50, 75, and 100 µM berberine for 24, 48, and 72 h reduced cell growth in a time- and concentration-dependent manner and arrested SKBR-3 cells in the G_{1} phase of the cell cycle by downregulating the expression of cyclin D1/E. Treatment with berberine stimulated apoptosis and DNA fragmentation, attenuated Bcl-2 expression, activated caspase 9/3 and PARP, indicating the involvement of the mitochondrial/caspase pathway and the downregulation of HER2/Pi3K/Akt signaling (Table 1).84 Berberine (10, 25, 50, 75, or 100 µM) induced cytotoxicity, decreased cell migration, invasion, and expression of MMP 2/9 and suppressed expression of Akt (protein and mRNA), NF-$\kappa$B, c-Jun, and activator protein 1 (AP-1) in MDA-MB-231 and MCF-7 cells (Table 1).85 Liposomal berberine accumulated in MCF-7 cell membranes and berberine liposomes (including targeted liposomes) reduced cell survival depending on berberine concentration (10, 20, 30, 40, and 50 µM) in MCF-7 and MCF-7 cancer stem cells

DOI: 10.14218/JERP.2021.00005 | Volume 00 Issue 00, Month Year 13

Jagetia G.C.: Anticancer activity of berberine J Explor Res Pharmacol...
Berberine treated MCF-7 (20, 40, 80, 120, and 160 µM) and MDA-MB-231 (10, 20, 40, 80, and 120 µM) cells had decreased cell viability depending on the concentration with IC_{50} values of 19.0 ± 0.2 µM and 85.5 µM, respectively, and decreased colony formation: MDA-MB-231 cells were more sensitive than MCF-7 cells. Berberine induced G_{1} arrest in MCF-7 cells but not in MDA-MB-231 cells. Microarray analysis revealed that berberine upregulated 1318 and downregulated 2079 genes in MCF-7 cells, whereas MDA-MB-231 cells showed an upregulation of 1662 and downregulation of 1044 genes. Gene Ontology (GO) analysis indicated that berberine altered the regulation of genes related to apoptosis, cell cycle, cell migration, and drug response. Tenfold more genes were regulated in MCF-7 cells compared to MDA-MB-231 cells. Quantitative (q)PCR analysis showed that berberine upregulated cyclin G1, and downregulated angiopoietin-like 4 (ANGPTL4) and colony stimulating factor 1 receptor (CSF1R)-related genes, including at the mRNA level, in MDA-MB-231 cells but not in MCF-7 cells. The mRNA expression of cytochrome P450 family 1 subfamily A member 1 (CYP1A1) and GADD45A was significantly upregulated, whereas that of CXCRI4 was downregulated in MCF-7 and MDA-MB-231 cells. Berberine treated (10, 25, 50, 75, and 100 µM) MCF-7 and MDA-MB-231 cells exhibited a decline in cell viability that was time- and concentration-dependent, as well as increased apoptosis and ROS production. Berberine activated proapoptotic JNK signaling, depolarized Δψm, reduced Bcl-2 expression, and increased Bax, caspase-3 activity, cytochrome c, and AIF release from mitochondria (Table 1).

Exposure of BT549 and MDA-MB-231 cells to 5, 10, 20, 40, and 80 µM berberine for different time periods increased cell proliferation in a concentration- and time-dependent manner with IC_{50} values of 16.575 ± 1.219 µg/mL and 18.523 ± 6.139 µg/mL, respectively. Berberine decreased colony formation, cell migration, and Bcl-2 expression, and increased apoptosis and DNA DSBs (γH2AX), caspase-3/9 activity, cytochrome c release, ligase 4, and Bax expression (Table 1). Berberine treatment arrested cell motility by decreasing transforming growth factor beta (TGF-β)1 and MMP-2 levels, without altering TGF-β2. In another study Hs578t triple-negative breast cancer cells treated with 25 and 50 µM berberine showed decreased IL8 expression, reduced invasiveness, and downregulated expression of EGFR, MEK, and ERK with reduced phosphorylation of MEK and ERK (Table 1).

MDA-MB-231 and MCF-7 cells treated with 20, 40, and 80 µM or 6.25, 12.5, 25, 50, and 100 µM berberine had reduced cell proliferation depending on the concentration and length of treatment, and increased apoptosis and necrosis. Berberine arrested cells in the G_{1} phase of the cell cycle, upregulated p21^{Cip1} and p27^{Kip1} mRNAs and proteins, increased p53 protein, phospho-p53Ser15, and GSK3β, and reduced expression of cyclin D1/E, CDK4/6, phospho-AktThr308, total Akt, e-Myc, EGFR, Akt, ERK1/2, and p38 kinase activity and phosphorylation. Berberine treated MDA-MB-231 (0, 3, 6, and 12 µM) and MDA-MB-231 (0, 6.25, 12.5, and 25 µM) cells showed a concentration-dependent reduction in cell proliferation. Berberine arrested MDA-MB-231 and MDA-MB-453 cells in the S- and G_{2}/M phases whereas MDA-MB-468 and BT-549 cells were arrested in the G_{1}/S phase of the cell cycle. The expression of cyclin A/D and CDK1/4 were reduced in MDA-MB-468 and BT-549 cells treated with berberine (Table 1).

Exposure of MCF-7 and MCF12A (non-tumorigenic epithelial cells) cells treated with 1 and 10 µM berberine had slower proliferation and cells arrested in the G_{1}/G_{2} phase of the cell cycle; however, when the berberine concentration was increased up to 100 µM, MCF7 cells exhibited cell death, but the MCF12A (non-tumorigenic) cells did not. Berberine elevated the protein expression of p53 and p21 in a time- and concentration-dependent manner in MCF-7 cells (Table 1). Berberine accumulated in the mitochondria of both cells at the higher concentration (10 or 100 µM), and the accumulation within the nucleolus was prominent after the transition to the nucleoplasm in MCF7 cells. Berberine increased nucleolar stress in MCF7 cells, as indicated by the loss of ribosomal protein (RP1L5 from the nucleolus and the nuclear aggregation of p53 protein. Treatment of MCF-7 and MDA-MB-231 cells with 25 or 50 µM berberine led to a reduction in cell survival, cell migration, invasion, and cell arrested in the G_{1}/M phase of the cell cycle. Berberine elevated miR-214-3p expression, Bax/Bcl-2 ratio, and secretin (SCT) expression at the mRNA and protein levels (Table 1).
Berberine had reduced cell viability and motility according to the concentration of berberine, and 20 mM berberine had the maximum effect on cell invasion inhibition in SiHa cells. Berberine reduced the activities of u-PA and MMP-2 and increased TIMP-2 expression in SiHa cells. Berberine treatment reduced TGF-β1, EMT, phosphorylation of p38, FAK, paxillin, NF-κB, and Src, as well as the expression of Snail-1, C23, and β-catenin (Table 1). Exposure of SiHa and CalXi cells to 150, 200, and 250 µM berberine concentration-dependently decreased cell viability, migration, and invasion. Berberine increased apoptosis, expression of Bax and caspase 3 and reduced Bcl-2 expression. Berberine repressed EMT in cells by reducing MMP-9, N-cadherin, and vimentin expression and increasing E-cadherin and keratin 17 (KRT17) expression (Table 1).

**Leukemia**

Exposure of promyelocytic leukemia HL-60 cells to 5, 10, 25, and 50 µg/mL berberine reduced cell viability and elevated apoptosis and intermembranous DNA fragmentation in a concentration-dependent manner, which was observed in cells treated with 20, 40, 60, and 120 µM berberine (Table 1). Berberine arrested cells in the G0/G1 phase up to 24 h, and downregulated mRNA and telomerase. In vitro studies on calf thymus DNA revealed that berberine complexed with DNA to form berberine DNA-complexes (Table 1). Treatment of HL-60 cells with 10, 50, and 100 µM berberine inhibited N-acetyltransferase (NAT) activity, 2-aminofluorenone (AF)-DNA adduct formation, and downregulated mRNA expression in a concentration-dependent manner, arrested cells in the G2/M phase up to 24 h, and downregulated mRNA and telomerase. In vitro studies on calf thymus DNA revealed that berberine complexed with DNA to form berberine DNA-complexes (Table 1).

**Prostate cancer**

Berberine (10–100 µM) treated DU145 and PC-3 (androgen-insensitive) and LNCaP (androgen-sensitive) prostate cancer cells had reduced cell proliferation and increased cell death depending on the concentration and length of exposure to berberine, whereas non-neoplastic PWE-1 human prostate cells remained almost unaffected. Berberine arrested DU145 cells in the G1 phase of the cell cycle, downregulated the expression of cyclins D1/E2 and CDK 2/4/6, and increased the expression of p21/Cip1 and p27/Kip1. Berberine induced apoptosis and fragmented cell DNA in DU145 and LNCaP cells, and increased Bax/Bcl-2 ratio, caspase 9/3 and PARP activation, and depolarized Δψm. Similar observations were reported in PC-3 cells exposed to 25, 50, 75, and 100 µM berberine, except that the increased ROS formation, the release of cytochrome c, and second mitochondria-derived activator of caspase (Smac)/direct inhibitor of apoptosis-binding protein with low pI (DIABLO) from mitochondria (Table 1). PC-3 and LNCaP cells treated with 5, 10, 20, 50, and 100 µM berberine experienced suppressed cell growth depending on length of treatment time and concentration, but no such effect was detected in normal human prostate epithelial WPE-1E cells. Berberine arrested the cells in the G0/G1 phase and induced apoptotic cell death by increasing Bax and caspase 3 activation (Table 1). LNCaP (p53+) cells were more sensitive than PC-3 (p53-) cells to berberine treatment.

LNCaP and PC-3 cells exposed to 20, 100, and 200 µM berberine for 24, 48, and 72 h experienced attenuated cell growth depending on the length of treatment and concentration, along with increased apoptosis and arrested cells in the G1 phase. Berberine blocked the expression of prostate-specific antigen and the activation of EGFR (Table 1). Treatment of LNCaP, LAPC-4, 22Rv1, C4-2B, and PC-3 cells with 1.56, 3.125, 6.25, 12.5, 25, 50, and 100 µM berberine reduced cell proliferation, and androgen receptor (AR)-positive cells (LNCaP and LAPC-4) were more sensitive than AR-negative cells. Berberine triggered apoptotic cell death in LNCaP cells depending on the concentration, and inhibited the transactivation of AR in AR-dependent and AR-independent cells to the same extent (Table 1). Berberine (5, 10, 20, and 50 µM) treated murine prostate cancer RM-1 cells exhibited a concentration-dependent reduced cell proliferation, increased DNA DSBs and apoptosis, and arrested cells in the G1 phase of the cell cycle. Berberine activated the p53-p21 cascade at a low concentration and arrested cells in the G1/M phase at a higher concentration (50 µM for 24 h) due to increased phosphorylation of ataxia-telangiectasia mutated (ATM)/checkpoint kinase 1 (Chk1). Treatment of PC3 human and RM-1 mouse prostate cancer cells with 5, 10, 20 or 50 µM berberine resulted in reduced cell viability depending on the concentration, and the arrest of PC3 cells in the G1/G0 (10 µM) or G2/M (50 µM) phase of the cell cycle. Treatment of LNCaP and PC-82 cells with 1,
5, 25, 50, and 100 µM berberine reduced cell proliferation and decreased cell viability in a concentration-dependent manner. Berberine increased apoptosis and programmed necrosis by increasing the release of cyclophilin-D (Cyp-D) from mitochondria and the translocation of p53 into the mitochondria in these cells, ultimately causing cytotoxicity (Table 1). Berberine (5, 20, 40, 60, and 80 µM) treated MG-63 cells showed a concentration-dependent reduction in cell proliferation. Berberine arrested cells in the G1 phase of the cell cycle, which was dependent on p53 expression and an elevation in p21 and p27, whereas p53 did not have any effect on G2/M cell cycle arrest. Berberine also reduced the levels of cyclin E depending on the concentration of berberine, whereas cyclin D1 was attenuated only at 50 µg/mL. Berberine triggered apoptosis in a concentration-dependent manner due to elevated levels of p53, Bax, p53 upregulated modulator of apoptosis (PUMA), and Fas in these cells. Berberine treatment induced DNA DSBs, as indicated by a concentration-dependent rise in γH2AX (Table 1).

### Osteosarcoma

Osteosarcoma cells, including U2OS, Saos-2, and HOS, treated with 1, 5, 10, 20, and 50 µg/mL berberine had a concentration- and time-dependent reduction in cell proliferation. Berberine arrested cells in the G1 phase of the cell cycle, which was dependent on p53 expression and an elevation in p21 and p27, whereas p53 did not have any effect on G2/M cell cycle arrest. Berberine also reduced the levels of cyclin E depending on the concentration of berberine, whereas cyclin D1 was attenuated only at 50 µg/mL. Berberine triggered apoptosis in a concentration-dependent manner due to elevated levels of p53, Bax, p53 upregulated modulator of apoptosis (PUMA), and Fas in these cells. Berberine treatment induced DNA DSBs, as indicated by a concentration-dependent rise in γH2AX (Table 1).

### Lung cancer

A549 human lung cancer cells treated with 2.5, 5.0, 10, 20, 40, and 80 µM berberine resulted in a concentration- and time-dependent suppression of cell proliferation, migration, and invasion, and cells arrested in the G1 phase of the cell cycle. Berberine inhibited the expression of cyclin B1, cAMP response element-binding protein (CREB), MAPK, MMP-2, uPA, NF-κB, cFos, cJun, and phosphorylation of Akt. Berberine treatment reduced the transcription of MMP-2 mRNA but upregulated TIMP-2 mRNA and protein expression. Berberine treated A549 and H1299 cells showed a concentration- (25, 50, 75, and 100 µM) and time-dependent reduction in cell proliferation and increase in apoptosis and DNA fragmentation. Berberine disrupted Δψm, attenuated Bcl-2 and Bcl-xL expression, and increased Bax, Bak, and caspase 3 activation (Table 1). H460 cells treated with 1.0, 1.5, and 10 µM berberine showed a concentration-dependent reduction in cell proliferation in a concentration-dependent manner.
growth with an IC50 of 5 µM and cells were arrested in the G0/G1 phase of the cell cycle. A549 cells treated with 20, 40, 80, and 160 µM berberine showed reduced cell invasion, migration, and EMT, as well as an inhibition of vimentin, Snail-1 and Slug with an increase in the expression of E-cadherin. A549 cells treated with 6.25, 12.5, 25, 50, and 100 µM berberine had reduced cell viability, increased ROS and apoptosis, and cells were arrested in the G0/G1 phase of the cell cycle in a concentration-dependent manner. Berberine increased the expression of p21 and reduced cyclin D1. Berberine (3.125, 6.25, 12.5, 25, 50 or 50 µM) increased p21WAF1 (at low concentrations), IL6, IL1β, TNF-α, p-pIκBα, and mTOR (at higher concentrations) and decreased NF-κB and cyclin A1/2/B1 expression, but had no effect on cyclin D1 expression (Table 1).

The accumulation of berberine in cells is important for its action, and one study has shown a two-to-threefold accumulation of berberine in H1650 and H1975 cells and cell organelles compared to normal BEAS-2 lung cells. A549 cells treated with 20, 40, 80, 100, 120, 140, 160, 180, and 200 µmol/mL berberine had reduced cell viability, increased ROS generation, and cells were arrested in the G0/G1 phase of the cell cycle. Berberine reduced Δψm and increased nuclear fragmentation along with mRNA and protein levels of p21, p53 and Bax, increased cytochrome c release and activated caspase 8/9/3, and decreased Bcl-xl, Bcl-2, TNF-α, COX-2, MMP-2, MMP-9 and HDAC 1/2/4 (Table 1). A549 cells exposed to 30, 60, 90, 150, and 200 µM berberine had a concentration and time-dependent reduction in cell proliferation and an increase in cell apoptosis. Berberine downregulated MMP-2, increased Bcl2/Bax signaling, and inhibited Janus kinase-2 (JAK-2), VEGF, NF-κB, and AP-1 proteins in A549 cells. A549 and PC9 cells treated with 20, 40, 60, 80, 100, 120, 140, and 160 µM berberine had reduced cell proliferation and colony formation through increased apoptosis. Berberine reduced Bcl2 and increased the expression of Bax and TF mRNA, which was followed by the downregulation of miR-19a in a concentration-dependent manner. This was followed by increased phosphorylation of JNK and p38MAPK (Table 1). NCI-H460, A549 and NCI-H1299 cells treated with 10, 20, 40 and 80 µM berberine had reduced cell proliferation and colony formation with IC50 values of 30.3 µM, 44.5 µM, and 43.8 µM, respectively. Berberine induced DNA DSBs and apoptosis, and downregulated DNA topoisomerase 2-beta (TOP2B) and SIN3 transcription regulator family member A (Sin3A) expression, and shortened the half-life of Sin3A in human NSCLC cells (Table 1).

Pancreatic cancer

Exposure of human pancreatic cancer cells BxPC-3 and pancreatic duct HPDEE6E7c7 cells to 10, 50, 100, 150, and 200 µM berberine resulted in a concentration and time-dependent decline in cell survival with an IC50 of 62.8 µM for the former, but the IC50 could not be determined for the latter, indicating that BxPC-3 cells were more sensitive to berberine than HPDEE6E7c7 cells (Table 1). Berberine (150 and 200 µM) increased apoptosis, activated caspase 3/7, and stimulated the release of AIF. Microarray analysis showed that berberine treatment upregulated 234 genes and downregulated 33 genes, which were related to BRCA1-mediated DNA damage response, G1/S and G2/M cell cycle checkpoint regulation, and p53 signaling. Berberine treatment of pancreatic cancer stem cells PANC-1 and MiaPaCa-2 resulted in IC50 values of 15 and 10 µM, respectively, and it also decreased the side population of cells. Berberine downregulated Sox2, POU class 5 homeobox 1 (POU5F1), and Nanog homeobox (NANOG) genes in both cells, but the NOTCH1 gene remained undetectable. PAN-1 and MiaPaCa-2 cells exposed to 1, 5, 7, 10, and 15 µM berberine had reduced cell proliferation, and cells were arrested in the G0 phase of the cell cycle, and apoptosis was triggered by ROS production (Table 1).

Pancreatic duct cells PANC-1 and MiaPaCa-2 treated with 0.15, 0.3, 1.5, 3, and 6 µM berberine showed inhibited cell proliferation and DNA synthesis, and delayed progression through the G1 phase of the cell cycle. Berberine reduced Δψm and intracellular ATP levels and increased the phosphorylation of AMPK at Thr172 and acetyl-CoA carboxylase (ACC) at Ser79. Berberine inhibited mTORC1 (phosphorylation of S6K at Thr389 and S6 at Ser235/236) and ERK activation (Table 1). PANC-1 cells treated with 1, 5, 7.5, 10, 15, and 30 µM of berberine had reduced cell proliferation and increased apoptosis in a concentration-dependent manner. Berberine also inhibited cell migration and decreased TNF-α expression (Table 1). RNA sequencing detected 7368 differentially-expressed genes, out of which 3726 genes were downregulated and 3642 genes were upregulated after berberine treatment. Berberine downregulated K-ras genes and upregulated the tumour suppressor CDKN2A gene. Berberine treatment also increased amino acids, nucleotides metabolism and glycolysis, but reduced citric acid cycle metabolites and damaged the mitochondria (Table 1).

Renal cancer

Treatment of ACHN human renal cancer cells with 10 and 20 µmol/L berberine significantly inhibited cell proliferation and expression of c-Fos (Table 1). However, berberine did not induce the cleavage of caspase proteins, indicating that berberine did not trigger apoptosis. G401 Wilms’ tumor cells treated with 5, 10, 20, and 50 µM berberine had reduced cell proliferation that was concentration-dependent, and upregulated mRNA and protein expression of p21 and p27. Berberine downregulated mRNA and protein expression of cyclin E, indicating that it interferes with the cell cycle. Berberine activated the phosphorylation of AMPK and T-ACC (a downstream target of AMPK) and increased the phosphorylation of mTOR and S6 kinase, as well as increased the expression of the tumor suppressor gene Wilms tumor gene on X chromosome (WTX) in G401 cells (Table 1).

Bladder cancer

The treatment of human bladder cancer T24 cells with 0.8, 8, 80, 800, and 1,600 µM berberine resulted in decreased arylamine N-acetyltransferase activity (overactivated in tumor cells) in a concentration-dependent manner (Table 1). BIU-87 and T24 cells exposed to 1, 5, 10, 25, 50, 75, and 100 µM berberine for 24, 48, and 72 showed inhibition of cell viability in a concentration- and time-dependent manner. Berberine arrested cells in the G0/G1 phase of the cell cycle and induced apoptosis by activating cleaved caspase-3/9 depending on the concentration. Similarly, berberine caused concentration- and time-dependent inhibition of H-Ras and c-fos mRNA and protein expression (Table 1). Treatment of T24 cells with 10, 25, and 50 µg/ml berberine concentration-dependently attenuated cell migration and invasion, in addition to causing the downregulation of both mRNAs and protein levels of heparanase, which is linked to tumor cell migration and invasion (Table 1).

Thyroid cancer

Treating the thyroid cancer cells 8505C and TPC1 with 1, 10, and
100 µM berberine caused a concentration-dependent growth inhibition with an IC₅₀ of 10 µM for both cell types, and cells were arrested in the G₀/G₁ phase of the cell cycle. Berberine upregulated p-27 and the effect was more pronounced in TPC1 than 8505 cells. FTC-133 and 8305C cells treated with 10, 25, 50, and 100 µM berberine for 24, 48, and 72 h resulted in a concentration- and time-related reduction in cell viability. Berberine induced apoptosis, DNA fragmentation, and activation of cleaved caspase 3. Berberine treatment resulted in cell cycle arrest and overexpression of p53 and p27 in both 8505 and TPC1 cells, and the effect was more pronounced in TPC1 cells (Table 1). TT cells treated with 0.4, 0.8, 1.6, 3.2 and 6.4 µg/mL berberine had decreased cell viability depending on the concentration and repressed the expression of PI3K, p-Akt/Akt, and Nrf2 (Table 1).

In vivo studies

Berberine has been tested for its anticancer activity in different animal models of cancer (Table 2). The in vivo anticancer activity of 2–12 mg/kg body weight berberine killed Ehrlich ascites tumor cells in tumor bearing mice in a dose-dependent manner and increased the average and mean survival time of tumor bearing mice (Table 2). Berberine reduced tumor growth, downregulated EGFR, and induced senescence. Berberine (50 mg/kg) treated nude mice xenografted with U87 human glioblastoma cells and treated with 50 and 100 mg/kg berberine showed inhibited tumor growth, downregulated p-AMPK, and reduced tumor weight. The A459 xenografts were more sensitive than the H1299 xenografted tumors. The A459 xenografts were more sensitive than the H1299 xenografted tumors. The A459 xenografts were more sensitive than the H1299 xenografted tumors. The A459 xenografts were more sensitive than the H1299 xenografted tumors.

100 µM berberine caused a concentration-dependent growth inhibition with an IC₅₀ of 10 µM for both cell types, and cells were arrested in the G₀/G₁ phase of the cell cycle. Berberine upregulated p-27 and the effect was more pronounced in TPC1 than 8505 cells. FTC-133 and 8305C cells treated with 10, 25, 50, and 100 µM berberine for 24, 48, and 72 h resulted in a concentration- and time-related reduction in cell viability. Berberine induced apoptosis, DNA fragmentation, and activation of cleaved caspase 3. Berberine treatment resulted in cell cycle arrest and overexpression of p53 and p27 in both 8505 and TPC1 cells, and the effect was more pronounced in TPC1 cells (Table 1). TT cells treated with 0.4, 0.8, 1.6, 3.2 and 6.4 µg/mL berberine had decreased cell viability depending on the concentration and repressed the expression of PI3K, p-Akt/Akt, and Nrf2 (Table 1).

In vivo studies

Berberine has been tested for its anticancer activity in different animal models of cancer (Table 2). The in vivo anticancer activity of 2–12 mg/kg body weight berberine killed Ehrlich ascites tumor cells in tumor bearing mice in a dose-dependent manner and increased the average and mean survival time of tumor bearing mice (Table 2). Berberine reduced tumor growth, downregulated EGFR, and induced senescence. Berberine (50 mg/kg) treated nude mice xenografted with U87 human glioblastoma cells and treated with 50 and 100 mg/kg berberine showed inhibited tumor growth, downregulated p-AMPK, and induced senescence. Berberine (50 mg/kg) treated athymic nude mice xenografted with U87 human glioblastoma cells and treated with 50 and 100 mg/kg berberine showed inhibited tumor growth, downregulated p-AMPK, and induced senescence. Berberine (50 mg/kg) treated athymic nude mice xenografted with U87 human glioblastoma cells and treated with 50 and 100 mg/kg berberine showed inhibited tumor growth, downregulated p-AMPK, and induced senescence.
### Table 2. Anticancer activity of berberine in various animal models

| Mouse Model | Berberine | Outcome | References |
|-------------|-----------|---------|------------|
| BALB/c | 2-12 mg/kg | Ehrlich ascites carcinoma | Increased average and mean survival time | 166,167 |
| BALB/c nude | 50 and 100 mg/kg | U87 human glioblastoma cells | Inhibited tumor growth, induced senescence, downregulated EGFR | 33 |
| BALB/c nude | 50 and 100 mg/kg | U87 human glioblastoma cells | Reduced tumor growth, upregulated p-AMPK, downregulated p-mTOR, LC3B, reduced Ki-67 positive cells | 34 |
| Athymic nude | 50 mg/kg | U87 human glioblastoma cells | Reduced tumor volume, hemoglobin level, mRNAs CD31, VEGFR2, ERK, p38, angiogenesis | 168 |
| BALB/c nude | 10 mg/kg | MCF-7CSC breast cancer cells | Inhibited tumor growth, nontoxic to blood cells | 86 |
| BALB/c nude | 10 mg/kg | MDA-MB-231 breast cancer cells | Reduced tumor volume, tumor weight | 82 |
| BALB/c nude | 100 mg/kg | MDA-MB-231 breast cancer cells | Reduced tumor growth, cell proliferation, Ki-67 labelling, upregulated caspase 9 activity | 89 |
| BALB/c nude | 0.1% (w/v) | TNBC 4T1 breast cancer cells | Reduced tumor growth, metastasis, G0/G1 arrest | 91 |
| BALB/c nu/nu | 10 mg/kg | BCG-823 human colon cancer cells | Reduced tumor growth, weight, p-Akt tumor tissue | 65 |
| BALB/c nu | 5, 10 or 20 mg/kg | BCG-823 human colon cancer cells | Reduced tumor growth, tumor weight, tumor cell proliferation, PCNA labelling, p-mTOR, p-p70S6K, p-Akt, p-JNK p-p38 increased autophagic death, LC3, Beclin-1 | 66 |
| BALB/c nu/nu | 10, 30, or 50 mg kg | LoVo colon cancer cells | Reduced tumor growth, tumor volume | 69 |
| BALB/c nude | 10 mg/kg | KM12C/shCtrl colon cancer cells | Reduced tumor growth, PCNA, Ki67, Cdc2, cMyc, β catenin, increased the p21WAF1/CIP1, RXRα | 169 |
| Nude | 50 and 100 mg/kg | HEC-1-A human endometrial carcinoma cells | Reduced tumor growth, cell migration and invasion in the mice lungs, increased transcription of miR-101 via activator protein 1, reduced COX-2/PGE2 signaling pathways | 170 |
| Athymic nude | 50, 100 and 200 mg/kg | A459 and H1299 lung cancer cells | Reduced tumor growth | 141 |
| BALB/c nude | 5 and 10 mg/kg | A459 lung cells | Reduced tumor growth | 143 |
| BALB/c nude | 50 mg/kg | RPMI-8266 multiple myeloma cells | Reduced tumor growth, increased mouse survival | 116 |
| Mouse | 10 mg/kg | B16 melanoma | Reduced tumor, tumor volume, tumor weight | 171 |
| BALB/c nude | 5 and 10 mg/kg | PC-3 and LNCaP prostate cancer cells | Reduced tumor growth, tumor volume, tumor weight, increased cleaved caspase 3, PARP activities | 119 |
| BALB/c | 12.5, 25 and 50 mg/kg | H22 mouse HCC cells | Reduced tumor growth and volume | 73 |
| BALB/c nu/nu | 10 mg/kg | MHCC-97L human HCC | Reduced tumor growth, lung metastasis, HIF-1α/VEGF signaling and expression of Id-1 | 172 |
| BALB/c nude | 10 mg/kg | SSC-4 tongue carcinoma cells | Reduced tumor growth, tumor volume, tumor mass | 173 |
| Nude | 5 and 10 mg/kg | C666-1 nasopharyngeal carcinoma cells | Reduced tumor growth, STAT-3 activation | 174 |

AMPK, AMP-activated protein kinase; Akt, protein kinase B; Cdc, cell division cycle; COX, cyclooxygenase; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; HCC, hepatocellular carcinoma; HIF, hypoxia-inducible factor; Id-1, inhibitor of differentiation/DNA binding; JNK, c-Jun N-terminal kinase; LC3, microtubule-associated protein 1A/1B-light chain 3; mTOR, mechanistic target of rapamycin; PARP, poly(ADP-ribose) polymerase; PCNA, proliferating cell nuclear antigen; PGE2, prostaglandin E2; STAT, signal transducer and activator of transcription; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; w/v, weight by volume.
and LNCaP prostate cancer cells and injected with 5 and 10 mg/kg body weight of berberine twice a week for four weeks experienced significantly reduced tumor growth rates, volumes, and weights. Berberine treatment significantly increased the activities of cleaved caspase 3 and PARP in the mice treated with 10 mg/kg berberine, indicating the role of apoptosis in tumor shrinkage (Table 2). The LANCaP tumors were more sensitive than PC3 tumors to berberine treatment. 119

H22 mouse HCC cells were transplanted into BALB/c mice, and mice that received 12.5, 25 and 50 mg/kg berberine had reduced tumor growth and volume that was dose-dependent. 79 The orthotopic model of MHCC-97L human HCC in BALB/c nu/nu mice treated with 10 mg/kg berberine every two days had effectively reduced tumor growth in the liver as well as the lung metastasis. Berberine treatment suppressed HIF-1α/VEGF signaling and expression of inhibitor of differentiation/DNA binding (Id-1), a key regulatory molecule for HCC development. 172 The BALB/c nude mice with a xenograft of SSC-4 tongue carcinoma cells were treated with 10 mg/kg of berberine, which resulted in reduced tumor mass, volume, and growth. 75 The athymic nude mice with a C666-1 human NPC xenograft and administered with 5 and 10 mg/kg berberine every two days did not show tumor development on 30 and 36 days, respectively. However, tumors started to appear 31 ± 5 and 37 ± 5 days after the animals received 5 and 10 mg/kg berberine, respectively. Tumorigenic growth was detected in three out of five mice in 10 mg/kg berberine treatment group, indicating that berberine inhibited the growth of C666-1 nasopharyngeal carcinoma in nude mice. Berberine suppressed the activation of STAT-3 in vivo (Table 2). 174

**Clinical studies**

There is only one double-blinded multicentre clinical trial that was conducted in China, where 0.3 g berberine or placebo tablets were given twice daily to colorectal cancer patients (18–75 years of age) who had more than six histologically confirmed colorectal adenomas, including tubular, tubulovillous, and villous, which were surgically removed six months prior to recruitment. The berberine group consisted of 553 patients, whereas the placebo group consisted of 555 patients. Analysis of 429 patients from the berberine group and 462 patients from the placebo group after two years revealed that 155 (36%) patients had recurrent adenomas in the berberine group, compared to 216 (47%) patients in the placebo group (unadjusted relative risk ratio for recurrence: 0.77, 95% confidence interval (CI) 0.66–0.91; p = 0.001). The patients in the berberine-treated group did not have colorectal cancers during follow-up. Six (1%) patients complained of constipation out of 446 patients in the berberine group, which was the only adverse side effect of berberine. No other serious adverse effects were reported. 175

**Toxicity studies**

The mice injected with berberine through intravenous (i.v.) and intraperitoneal (i.p.) routes revealed LD₅₀ values of 9.0386 and 57.6103 mg/kg, respectively. However, it was not possible to determine LD₅₀ for the intragastric (i.g.) route since only 30% of deaths were recorded. 176 Berberine i.p. administration in rats revealed a LD₅₀ of 205 mg/kg; however, the administration of 50 mg/kg caused diarrhea in 40% of rats. The LD₅₀ in mice was 23 mg/kg following i.p. injection, whereas it was 329 mg/kg after oral administration. 177 Developmental toxicity of berberine was studied in pregnant rats at 6–20 days of gestation (GD) and mice at 6–17 GD. Rats were given 3,625, 7,250, or 14,500 ppm and mice were given 3,500, 5,250, or 7,000 ppm in their feed. The berberine did not cause the maternal death of any rats or mice, and the lowest observed adverse effect level (LOAEL) for rats was 7,250 ppm (531 mg/kg/day) and for mice was 5,250 ppm (841 mg/kg/day). Thirty-three percent of female mice died, and surviving animals drank more water. There was a reduction in fetal body weights of both rats and mice and no other adverse effects were seen. 178

Clinically diabetic patients receiving 500 mg berberine three times a day for 13 weeks exhibited transient gastrointestinal side effects including constipation, diarrhea, abdominal pain, and flatulence with no obvious alterations in liver enzymes and creatinine levels. 179 Four out of 12 cardiac patients receiving 0.2 mg/kg berberine infusion for 30 min exhibited ventricular tachycardia with torsade de pointes as an adverse side effect of berberine. 180 Administration of berberine in infants caused kernicterus with glucose-6-phosphate-dehydrogenase (G6PD) deficiency and displacement of bilirubin from binding proteins. 181,182 Though berberine has been reported to be safe clinically, it should not be given to pregnant women, breastfeeding mothers, or G6PD-deficient neonates. Individuals with severe gastrointestinal disorders should also not be given berberine to avoid further complications.

**Mechanism of action**

Berberine employs multiple putative mechanisms to trigger cytotoxicity in various cancer cells (Fig. 2–4). One of the most important mechanisms of action of berberine is the acceleration of ROS formation in various cancer cells, which eventually leads to the stimulation of various pathways to kill cells. 30–32,42–44,45,57,58,59,60,68,69,78,88,91,114,145,147,161 Berberine is able to kill a variety of cancerous cells by triggering apoptosis (Fig. 2) which may be: (1) ROS-dependent, (2) Fas-dependent, (3) p53-dependent, or (4) p53-independent. The acceleration of ROS formation leads to alteration in the mitochondrial membrane permeability and increased Ca²⁺ release, which subsequently activates AIF release from the mitochondria that leads to caspase-independent apoptosis by berberine (Fig. 2). 42,44,59,88,151 The release of cytochrome c after berberine treatment also leads to apoptotic cell death by subsequent activation of Apaf-1, which causes the formation of apoptosomes and activation of caspase 9/7/3. Additionally, cytochrome c release is also mediated by the entry of Bcl2 family of proteins, especially BAX, into the mitochondria to trigger apoptosis. 30–32,35,42–44,53,55,59,71,86,88,89,114,147 The triggering of DNA damage (DNA fragmentation, DNA strand breaks) and ER stress by berberine also induces apoptotic cell death leading to suppression of Bcl2 and BclXL, and activation of tBid, BAX and BAK. 30–33,39,41,43,44,84,85,89,97,99,100,102,103,105,107,112,117,122,129,135,141,150,162 The secretion of Smac/DIABLO by the mitochondria after berberine treatment and subsequent activation of XIAP also induces cell death by apoptosis. 113,114,115 Berberine is also able to trigger the extrinsic pathway of apoptosis stimulated by TRAIL, FADD, FASL and TNFRs that activates caspases 8/7/3 and PARP. 30,31,37,41,43,44,48,53,55,59,61,65,69,99,112,113,117,136,138,155 Berberine stimulates necrosis by elevating the release of Cyp-D from mitochondria and promoting the translocation of p53 into mitochondria (Fig. 2). Berberine induces autophagy (Fig. 2) by upregulating LC3B-II and alleviating the SQSTM1/P62 proteins, and by converting LC3-I into LC3-II in various cell types (Fig. 2). 35,37,54

Berberine arrests cells in the G₀/G₁ phase of the cell cycle by negatively altering various cyclins and CDKs, and upregulating
CDK inhibitors (Fig. 2), which would also contribute to cell death by apoptosis. Berberine also arrests cell in the G₂/M phase of the cell cycle in some of the cell lines by activating Cdc2 (p-Cdc2; Tyr15) and suppressing p-histone as well as Cdc2 and Cdc25 expression.

Regarding the formation of complexes with berberine and DNA, polyadenylic acid (poly-A) has a stronger affinity to bind to berberine than poly U and poly C, which may contribute to its anticancer activity and neoplastic cell death (Table 1).

Berberine alters various cell signaling pathways to exert its anticancer activity in various neoplastic cells. EGF increases the clonogenic potential of cells by triggering cell proliferation, and the suppression of EGFR by berberine plays an important role in reducing cell proliferation by inhibiting downstream targets such as Akt, MEK, and ERK1/2, and their phosphorylation levels. VEGF is involved in angiogenesis, which is upregulated in different cancers due to various oncogenic stimuli including hypoxia, and berberine attenuates its expression along with VEGFR-2, reducing angiogenesis in various types of neoplasia. PI3K/Akt and MAPK (RAF/MEK/ERK) signaling pathways play a crucial role in normal gene expression and cell proliferation, and are linked to HER-2, EGFR, and various nuclear transcription factors. Berberine downregulates HER2/PI3K/Akt, EGFR-ErbB2/PI3K/Akt, and RAF, MEK, and ERK signaling pathways to exert its anticancer effect (Tables 1, 2).

mTOR controls cell division, apoptosis, and autophagy by participating in multiple signaling pathways, and its activation increases cell proliferation, gene transcription, protein synthesis, and immune cell differentiation in cancer. It also plays a crucial role in the metabolism of cancer cells. The suppression of mTOR activation in different cell lines by berberine is also one of its anticancer mechanisms of action.

The Wnt/β-catenin signaling pathway is involved in cell adhesion and its activation is linked to cell migration and invasion (metastasis), and berberine inhibits the activation of the Wnt/β-catenin signaling and reduces cell migration and invasion.
creased E-cadherin and decreased N-cadherin expression, and attenuated TGF-β. The P38/MAPK signaling pathway is crucial not only in Wnt/β-catenin signaling but also in EMT (Fig. 3). Berberine negatively alters N-cadherin, fibronectin, vimentin, ERK1/2, PI3K/Akt, Ras-Raf-ERK, MMP-9, PDGFRβ, COL1A2, Snail-1, and Slug to attenuate EMT. NF-κB and STAT-3 activation lead to an increase in cell survival, inflammation, and reduction in apoptosis, and they are overexpressed in the majority of cancerous cells. Berberine suppressed IKK/NF-κB and STAT3 activation, which seems to contribute to its anticancer effect in various cells.

COX-2 is also overexpressed in most cancer cells and its upregulation promotes tumour cell growth. Berberine inhibited COX-2 overexpression in different cell types to reduce their proliferation and growth rates.

Additionally, berberine interacts with numerous other targets to exert its anticancer effects (Fig. 4). It has been shown to suppress Jak-2, miR-19a, MMP 1, 2 & 16, CD133, n-myc, Sox2, Notch2, Nestin, IL-18, IL-1β, Mcl-1, FAK, pJNK, Nrf2, Rho GTPases, EBNA1, CC7, CXC4, c-IAP1, p70S6K, miR-21, ACC, ACL, FASN, SREBP-1, SCAP, PLA2, SP1, CCND1, E2F1, PTTG1, Skp2, p4EBP1, VASP, ANGPTL4, CSF1R, TGF-β1, p38 kinases, AP-1, hTERT, c-Fos, E6 and E7, HDAC1/2/4, HPV-18 E7, SMAD4, TIMP-2, paxillin, Src, C23, EZH2, BMP7, NODAL, RAD51, GLUT1, homologous recombination DNA repair, NANOG, POU5F1, ATP, lactate dehydrogenase A, HK2, GSH, NADPH, MRPL48, TUFM, PTC3. Berberine has also been shown to elevate PUMA, Cyp-D, EndoG, ER kinase, ETB-2, GRP-78, IHHBP, C/EBP, DNA damage-inducible gene 153, Rb, GADD153, GADD45α, KLF6, ATF3, FoxO3a, Wee1, 14-3-3σ, ATM/Chk1, Beclin-1, ULK-1, AMPK, Wilf-1, TCF-4, miR-145, miR-101, miR-155, miR-23a, miR-214-3p, CCNG1, CYP1A1, KRT17, c-Jun, NAG-1, ITGβ4, PDCD4, CD4KN2A, GSK3β and citrate synthase.

Future directions

It will be purposeful to investigate various molecular targets of berberine in vitro and in vivo by cellular thermal shift assay, proteomic profiling, RNA sequencing, microarray analysis, Gene ontology analysis and MALDI-TOF in future. Future studies should also be directed to investigate the toxic profile of berberine in hu-
Jagetia G.C.: Anticancer activity of berberine

Man volunteers to establish its safety after prolonged treatment. More clinical trials in different cancer types need to be conducted in future to firmly establish the chemotherapeutic potential of berberine in cancer treatment in clinical condition.

Fig. 4. Many targets participate in the cytotoxic action of berberine in various neoplastic cells. Red: upregulation. Blue: downregulation. ACC, acetyl-CoA carboxylase; ACL, adenosine triphosphate citrate lyase; AMPK, AMP-activated protein kinase; AP, activator protein; ATF, activating transcription factor; ATM, ataxia telangiectasia mutated; ATP, adenosine triphosphate; BMP, bone morphogenetic protein; C/EBP, CCAAT/enhancer-binding protein; CCR, C-C chemokine receptor type; CDK, cyclin-dependent kinase; COX, cyclooxygenase; CXCR, C-X-C motif chemokine receptor; DDIG, DNA damage-inducible gene; EBNA1, Epstein–Barr nuclear antigen 1; ERK, extracellular-regulated kinases; ETIF, eukaryotic translation initiation factor; FAK, focal adhesion kinase; FASN, fatty acid synthase; FoxO3a, forkhead box O3a; GADD, growth arrest and DNA damage-inducible genes; GSH, glutathione; HDAC, histone deacetylase; hERG, human ether-a-go-go-related gene; HICBP, immunoglobulin heavy chain binding protein; IL, interleukin; JNK, c-Jun N-terminal kinase; LDH, lactate dehydrogenase; MMP, matrix metalloproteinase; MRP, mitochondrial ribosomal protein; NADPH, nicotinamide adenine dinucleotide phosphate hydrogen; NAG, nonsteroidal anti-inflammatory drug activated gene; Nestin, neuroectodermal stem cell marker; Notch, neurogenic locus notch homolog protein; PTG, pituitary tumor transforming gene; SCAP, SREBP cleavage-activating protein; Skp, S-phase kinase-associated protein; SREBP, sterol regulatory element-binding protein; STAT, signal transducer and activator of transcription; TCA, T-cell factor; TUFM, Tu translation elongation factor; VASP, vasodilator-stimulated phosphoprotein; Wif, Wnt inhibitory factor.
Conclusions

Berberine triggers a cytotoxic effect in various cancer cells of different tissue origins as well as mouse/xenograft human tumor models, indicating its potential as an anticancer agent. Berberine is able to upregulate or downregulate several cellular proteins to kill various cancer cells. Berberine accelerates ROS formation in tumor cells by triggering both Fas and mitochondrion-mediated caspase-dependent and caspase-independent apoptosis, necrosis, and autophagy to kill cells. Berberine arrests cells in G0/G1, S- and G2/M phases, indicating that it can act at any stage of the cell cycle by suppressing cyclins and CDKs, and upregulating p53, p21Cip1 and p27Kip1. The cytotoxic effect of berberine is also due to its ability to modulate various cell signaling pathways including Wnt/b-catenin, mTOR, Ras-Raf-ERK, HER2/Ptck/Akt, EGFR-ErbB2/Ptck/Akt, INK, ATM/Chk1, p53, NF-kB, and COX-2/PGIE. A single clinical trial has shown improvement in gastric cancer patients with berberine. Clinically, berberine has exerted adverse effects in the form of constipation, diarrhea, abdominal pain, flatulence, and ventricular tachycardia with tordase de pointes in humans. Pregnant mothers should not be given berberine as it has shown adverse effects in preclinical models. There is a need to study the toxic profile of berberine more thoroughly in preclinical and clinical conditions to prove its safety after long term use in humans.

Acknowledgments

The author wishes to acknowledge the encouragement and patience of his wife Mrs. Mangla Jagetia during the preparation of this manuscript.

Conflict of interest

The author has no conflict of interest statement to declare.

Author contributions

This study is the sole work of GC Jagetia.

References

[1] Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2021. CA Cancer J Clin 2021. doi:10.3322/caac.21660.
[2] Mathur P, Sathishkumar K, Chaturvedi M, Das P, Sudarshan KL, Santihpan S, et al. Cancer Statistics, 2020: Report From National Cancer Registry Programme, India. JCO Glob Oncol 2020;6:1063–1075. doi:10.1200/GO.20.00122.
[3] WHO. WHO report on cancer: setting priorities, investing wisely and providing care for all. Geneva: World Health Organization; 2020. Available from: https://apps.who.int/iris/handle/10665/330745. Accessed March 05, 2021.
[4] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021. doi:10.3322/caac.21660.
[5] Simoens S, van Harten W, Lopes G, Vulto A, Meier K, Wilking N. What happens when the cost of cancer care becomes unsustainable? Eur Oncol Haematol 2017;13:108–113. doi:10.17925/EOH.2017.13.02.108.
[6] Newman DJ, Cragg GM. Natural Products as Sources of New Drugs from 1981 to 2014. J Nat Prod 2016;79(3):629–661. doi:10.1021/acs.jnatprod.5b01055.
[7] Sun Y, Xun K, Wang Y, Chen X. A systematic review of the anticancer properties of berberine, a natural product from Chinese herbs. Anticancer Drugs 2009;20(9):757–769. doi:10.1097/CAD.0b0133a328330d95b.
[8] Chen XW, Di YM, Zhang J, Zhou ZW, Li CG, Zhou SF. Interaction of herbal compounds with biological targets: A case study with berberine. Sci World J 2012;2012:708292. doi:10.1100/2012/708292.
[9] Singh HB, Bharati KA. Handbook of Natural Dyes and Pigments. New Delhi: Woodhead Publishing India Pvt Limited; 2015. doi:10.1016/C2014-0-02803-1.
[10] Pavelka S, Smekal E. The fluorescence properties of protoberberine and tetracyanodihydroprotoberberine alkaloids. Collect Czech Chem Commun 1976;41(10):3157–3169. doi:10.1135/ccs19763157.
[11] Domingo MP, Pardo J, Cebolla V, Galvez EM. Berberine: a fluorescent alkaloid with a variety of applications from medicine to chemistry. Mini Rev Org Chem 2010;7(7):345–390. doi:10.2174/157019310792464455.
[12] Nadkarni KM. Indian Materia Medica. 3rd edition. Bombay: Popular Prakashan; 1982.
[13] Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. New Delhi: National Institute of Science Communication and Information Resources; 2002.
[14] Chander V, Aswal JS, Dobhal R, Uniyal DP. A review on pharmacological potential of Berberis; an active component of Himalayan Berberis Glossary of Indian Medicinal Plants.aristata. J Phytopharm 2017;8(1):53–58.
[15] Li Z, Geng YN, Jiang JD, Kong WJ. Antioxidant and anti-inflammatory activities of berberine in the treatment of diabetes mellitus. Evid Based Complement Altern Med 2014;2014:289264. doi:10.1155/2014/289264.
[16] Yin J, Xing H, Ye J. Efficacy of berberine in patients with type 2 diabetes mellitus. Metabolism 2008;57(5):712–717. doi:10.1016/j.metabol.2008.01.013.
[17] Singh A, Duggal S, Kaur N, Singh J. Berberine: Alkaloid with wide spectrum of pharmacological activities. J Nat Prod 2010;3:64–75.
[18] Peng WH, Lo KL, Lee YH, Hung TH, Lin YC. Berberine produces antidepressant-like effects in the forced swim test and in the tail suspension test in mice. Life Sci 2007;81(11):933–938. doi:10.1016/j.lfs.2007.08.003.
[19] Peng L, Kang S, Yin Z, Jia R, Song X, Li L, et al. Antibacterial activity and mechanism of berberine against Streptococcus agalactiae. Int J Clin Exp Pathol 2015;8(5):5217–5223.
[20] Hu PF, Chen WP, Tang J, Bao JP, Wu LD. Protective effects of berberine in an experimental rat osteoarthritis model. Phytother Res 2011;25(6):878–885. doi:10.1002/ptr.3539.
[21] Chang W, Li K, Guan F, Yao F, Yu Y, Zhang M, et al. Berberine pre-treatment confers cardioprotection against ischemia-reperfusion injury in a rat model of type 2 diabetes. J Cardiovasc Pharmacol Ther 2016;21(5):486–494. doi:10.1177/1070424815627873.
[22] Cai Z, Wang C, Yang W. Role of berberine in Alzheimer’s disease. Neuropsychiatr Dis Treat 2016;12:2509–2520. doi:10.2147/NDDT.S114846.
[23] Zhao GL, Yu LM, Gao WL, Duan WX, Jiang B, Liu XD, et al. Berberine protects rat heart from ischemia/reperfusion injury via activating AKT2/STAT3 signaling and attenuating endoplasmic reticulum stress. Acta Pharmacol Sin 2016;37(3):354–367. doi:10.1038/aps.2015.136.
[24] Liu DQ, Chen SP, Sun J, Wang XM, Chen N, Zhou YQ, et al. Berberine protects against ischemia-reperfusion injury: A review of evidence from animal models and clinical studies. Pharmacol Res 2019;148:104385. doi:10.1016/j.phrs.2019.104385.
[25] Hu Y, Elhi EA, Kittelsrud J, Ronan P, Munger K, Downey T, et al. Lipid-lowering effect of berberine in human subjects and rats. Phytomedil
in vitro migration and invasion of human SCC-4 tongue squamous cancer cells through the inhibitions of FAK, IKK, NF-κB, u-PA and MMP-2 and -9. Cancer Lett 2009;279(2):155–162. doi:10.1016/j.canlet.2009.01.033.

26. Tang F, Wang D, Duan C, Huang D, Wu Y, Chen Y, et al. Berberine inhibits metastasis of nasopharyngeal carcinoma 5-BF cells by targeting rho kinase-mediated ezrin phosphorylation at threonine 567. J Biol Chem 2009;284(24):27456–27466. doi:10.1074/jbc.M109.037795.

27. Huang D, Wang W, Feng Z, Wang L, Chen Y, et al. Berberine inhibits the invasion and metastasis of nasopharyngeal carcinoma cells through Ezrin phosphorylation. J Cent South Univ (Medical Sci) 2011;36(7):616–623. doi:10.3969/j.issn.1672-7347.2011.07.006.

28. Li Ch, Wu Df, Ding H, Zhao Y, Zhou Ky, Xu DF. Berberine hydrochloride impact on physiological processes and modulation of twist levels in nasopharyngeal carcinoma CNE-1 cells. Asian Pacific J Cancer Prev 2014;15(4):1851–1857. doi:10.7314/APCP.2014.15.8.

29. Tsang CM, Lai EPW, Di K, Cheung PY, Hau PM, Ching YY, et al. Berberine inhibits G2 arrest and apoptosis in high doses but induces G2 arrest and apoptosis at high doses in human cancer cells. Int J Mol Med 2009;24(1):131–138. doi:10.3892/imj_00000216.

30. Wang C, Wang H, Zhang Y, Guo W, Long C, Wang J, et al. Berberine inhibits the proliferation of human nasopharyngeal carcinoma cells via an Epstein-Barr virus nuclear antigen 1-dependent mechanism. Oncol Rep 2017;37(4):2109–2120. doi:10.3892/or.2017.5489.

31. Mishan MA, Ahmadianka N, Matin MM, Heirani-Tabas A, Shahriyari M, Bidkhihi HR, et al. Role of berberine on molecular markers involved in migration of esophageal cancer cells. Cell Biol Mol Biol 2015;61(8):37–43. doi:10.14715/cmbm.2015.1.6.8.

32. Jiang SX, Qi B, Yao WJ, Gu CW, Wei XF, Zhao Y, et al. Berberine displays antitumor activity in esophageal cancer cells in vitro. World J Gastroenterol 2017;23(14):2511–2518. doi:10.3742/wjg.v23.i14.2511.

33. Chen X, Liu Y, Qi B, Gu C, Wei X, Guo L, et al. MicroRNA-212 facilitates the motility and invasiveness of esophageal squamous cancer cells. Mol Med Rep 2019;20(4):3633–3641. doi:10.3892/mmr.2019.10647.

34. Seo YS, Yim MJ, Kim BH, Kang KR, Lee SY, Oh JS, et al. Berberine-induced anticancer activities in FaDu head and neck squamous cell carcinoma cells. Oncol Rep 2015;34(6):3025–3034. doi:10.3892/or.2015.4312.

35. Zhang B, He J, Xue K. Berberine induces autophagy, apoptosis and modulates miR-155 in head and neck squamous carcinoma cells. Acta Pol Pharm Drug Res 2020;77(3):485–494. doi:10.32383/appdr/123018.

36. Hsu WH, Hsieh YS, Kuo HC, Teng CY, Huang HI, Wang CJ, et al. Berberine induces apoptosis in SW620 human colonic carcinoma cells through generation of reactive oxygen species and activation of JNK/p38 MAPK and Fasl. Arch Toxicol 2007;81(1):719–728. doi:10.1007/s00204-006-0169-y.

37. Pyanuch R, Sukhtantha M, Wandeeg G, Baek SJ, Berberine, a natural isoquinoline alkaloid, induces NAG-1 and ATF3 expression in human colorectal cancer cells. Cancer Lett 2007;258(2):230–240. doi:10.1016/j.canlet.2007.09.007.

38. Lin JP, Yang JS, Wu CC, Lin SS, Hsieh WT, Lin ML, et al. Berberine induced down-regulation of matrix metalloproteinase-1, -2 and -9 in human gastric cancer cells (SNU-5) in vitro. In Vivo 2008;22(1):223–230.

39. Park JJ, Seo SM, Kim EJ, Lee YI, Ko YG, Ha J, et al. Berberine inhibits human colon cancer cell migration via AMP-activated protein kinase-mediated downregulation of integrin β1 signaling. Biochem Biophys Res Commun 2012,426(4):461–467. doi:10.1016/j.bbrc.2012.08.091.

40. Chidambaram Murthy KN, Jayaprakash G, Patil BS. The natural alkaloid berberine targets multiple pathways to induce cell death in cultured human colon cancer cells. Eur J Pharmacol 2012;688(1–3):14–21. doi:10.1016/j.ejphar.2012.05.004.

41. Wu K, Yang Q, Mu Y, Zhou L, Liu Y, Zhou Q, et al. Berberine inhibits the proliferation of colon cancer cells by inactivating Wnt/beta-catenin signaling. Int J Oncol 2011;39(2):291–298. doi:10.3892/ijo_2011_0537.

42. Xu LN, Lu BN, Hu MM, Xu YW, Han Q, Yi Y, et al. Mechanisms involved in the cytotoxic effects of berberine on human colon cancer HCT-8 cells. Biocell 2012;36(3):113–120.

43. Mao L, Chen Q, Gou Y, Xu X, Xie Y, Zhang W, et al. Berberine decelerates glucose metabolism via suppression of mTOR-dependent HIF-1α protein synthesis in colon cancer cells. Oncol Rep 2018;39(5):2436–2442. doi:10.3892/or.2018.6318.
Kim JB, Yu JH, Ko E, Lee KW, Song AK, Park SY, 
Kim JB, Lee KM, Ko E, Han W, Lee JE, Shin I, 
Chuang TY, Wu HL, Min J, Diamond M, Azziz R, Chen YH. Berberine inhibits the growth of Anoikis-resistant MCF-7 and MDA-MB-231 breast cancer cell lines by inducing cell cycle arrest. Phytomedicine 2010;17(6):436–440. doi:10.1016/j.phymed.2009.08.012.

Su K, Hu P, Wang X, Kuang C, Xiang Q, Yang F, et al. Tumor suppressor berberine binds VASP to inhibit cell migration in basa-like breast cancer. Oncotarget 2016;7(29):45849–45862. doi:10.18632/oncotarget.9968.

Chou HC, Lu YC, Cheng CS, Chen YW, Lyu PC, Lin CW, et al. Proteomic and redox-proteomics analysis of berberine-induced cytotoxicity in breast cancer cells. J Proteomics 2012;75(11):3158–3176. doi:10.1016/j.jprot.2012.03.010.

Kuo HP, Chuang TC, Tsai SC, Tseng HH, Hsu SC, Chen YC, et al. Berberine, an isoquinoline alkaloid, inhibits the metastatic potential of breast cancer cells via Akt pathway modulation. J Agric Food Chem 2012;60(38):9649–9658. doi:10.1021/jf302832n.

Ma X, Zhou J, Chuang CX, Li YY, Li N, Ju RJ, et al. Modulation of drug-resistant membrane and apoptosis proteins of breast cancer stem cells by targeting berberine liposomes. Biomaterials 2013;34(18):4452–4465. doi:10.1016/j.biomaterials.2013.02.066.

Wen CJ, Wu LX, Fu LL, Yu Y, Zhang YW, Zhang X, et al. Genomic screening for targets regulated by berberine in breast cancer cells. Asian Pacific J Cancer Prev 2013;14(10):6089–6094. doi:10.7314/APCP.2013.14.10.6089.

Kuo HP, Chuang TC, Tsai SC, Tseng HH, Hsu SC, Chen YC, et al. Berberine, an isoquinoline alkaloid, inhibits the metastatic potential of breast cancer cells via Akt pathway modulation. J Agric Food Chem 2012;60(38):9649–9658. doi:10.1021/jf302832n.

Tak J, Sabarwal A, Shyanti RK, Singh RP. Berberine enhances posttranslational modification of Akt. Mol Cell Biochem 2019;458(1-2):49–59. doi:10.1007/s11010-019-03529-4.

Ma X, Zhou J, Chuang CX, Li YY, Li N, Ju RJ, et al. Modulation of drug-resistant membrane and apoptosis proteins of breast cancer stem cells by targeting berberine liposomes. Biomaterials 2013;34(18):4452–4465. doi:10.1016/j.biomaterials.2013.02.066.

Wen CJ, Wu LX, Fu LL, Yu Y, Zhang YW, Zhang X, et al. Genomic screening for targets regulated by berberine in breast cancer cells. Asian Pacific J Cancer Prev 2013;14(10):6089–6094. doi:10.7314/APCP.2013.14.10.6089.

Kuo HP, Chuang TC, Tsai SC, Tseng HH, Hsu SC, Chen YC, et al. Berberine, an isoquinoline alkaloid, inhibits the metastatic potential of breast cancer cells via Akt pathway modulation. J Agric Food Chem 2012;60(38):9649–9658. doi:10.1021/jf302832n.

Ma X, Zhou J, Chuang CX, Li YY, Li N, Ju RJ, et al. Modulation of drug-resistant membrane and apoptosis proteins of breast cancer stem cells by targeting berberine liposomes. Biomaterials 2013;34(18):4452–4465. doi:10.1016/j.biomaterials.2013.02.066.

Wen CJ, Wu LX, Fu LL, Yu Y, Zhang YW, Zhang X, et al. Genomic screening for targets regulated by berberine in breast cancer cells. Asian Pacific J Cancer Prev 2013;14(10):6089–6094. doi:10.7314/APCP.2013.14.10.6089.
Jagetia G.C.: Anticancer activity of berberine

J Explof Res Pharmacol

stream signaling to induce growth arrest and apoptosis in cervical cancer cells. Mol Cancer 2011;10:39. doi:10.1186/1476-4598-10-39.

[100] Saha SK, Khuda-Bukhsh AR. Berberine alters epigenetic modifications, disrupts microtubule network, and modulates HPV-18 E6/E7 oncoproteins by targeting p53 in cervical cancer cell Hela: A mechanistic study including molecular docking. Eur J Pharmacol 2015;744:132–146. doi:10.1016/j.ejphar.2014.09.048.

[101] Jagetia GC, Rao SK. Isoquinoline alkaloid berberine exerts its anti-neoplastic effect by inducing molecular DNA damage in hela cells: A comet assay study. Biol Med 2015;7(1):1000223. doi:10.11247/jpt/8369-1000223.

[102] Jagetia GC, Rao SK. Berberine chloride, an isoquinoline alkaloid, induces cytotoxicity in cultured Hela cells. Adv Biotechnol Biochem 2017:2;120. doi:10.29172/2574-7285.000002.

[103] Chu SC, Yu CC, Hsu LS, Chen KS, Su MY, Chen PN. Berberine reverses Doxorubicin-mediated cells apoptosis by upregulation of miR-24-3p in acute myeloid leukemia cells. Mol Med Rep 2020;26(1A):227–242. doi:10.3892/mmr.2019.10261.

[104] Liu C, Sun L, Zhang X, Liu A, Liu S, Zhang L, Wu B, et al. Berberine induces p53-dependent cell cycle arrest and apoptosis in human prostate cancer cells. Mol Cancer Ther 2008;6(5):2185–2195. doi:10.1158/1535-7163.MCT-08-0985.

[105] Li J, Zou Y, Pei M, Jiang Y. Berberine inhibits the Warburg effect through miR-145/PMPP16 axis in vitro. J Ovarian Res 2021;14(1):4. doi:10.1186/s13238-021-00542-2.

[106] Li J, Zou Y, Pei M, Jiang Y. Berberine inhibits the Warburg effect through miR-145/PMPP16 axis in vitro. J Ovarian Res 2021;14(1):4. doi:10.1186/s13238-021-00542-2.
Park SH, Sung JH, Chung N. Berberine diminishes side population
Chen J, Huang X, Tao C, Wang L, Chen Z, Li X, Chen QQ, Shi JM, Ding Z, Xia Q, Zheng TS, Ren YB, Li J, Liu F, Jiang S, Liu J, Chen X, Zhang S, Kalaiarasi A, Anusha C, Sankar R, Rajasekaran S, John Marshal J, Muc-
Peng PL, Hsieh YS, Wang CJ, Hsu JL, Chou FP. Inhibitory effect of ber-
Zhu Y, Ma N, Li HX, Tian L, Ba YF, Hao B. Berberine induces apoptosis
Yuan ZW, Leung ELH, Fan XX, Zhou H, Ma WZ, Liu L, Kumar R, Awasthi M, Sharma A, Padwad Y, Sharma R. Berberine in-
Qi HW, Xin LY, Xu X, Ji XX, Fan LH. Epithelial-to-mesenchymal transi-
Sung JH, Kim JB, Park SH, Park SY, Lee JK, Lee HS, Brazil J Med Biol Res 2015;48(2):111–119. doi:10.1590/1414-431X201404293.
[154] Ming M, Sinnett-Smith J, Wang J, Soares HP, Young SH, Eibl G, et al. Dose-dependent AMPK-dependent and independent mecha-
isms of berberine and metformin inhibition of mTORC1, ERK, DNA synthesis and proliferation in pancreatic cancer cells. PLoS One 2014;9(12):e114573. doi:10.1371/journal.pone.0114573.
[153] Liu J, Luo X, Guo R, Jing W, Lu H. Cell metabolism reveals berberine-
ihibits pancreatic cancer cell viability and metastasis by regulating
citrate metabolism. J Proteome Res 2020;19(9):3825–3836. doi:10.1021/acs.jproteome.0c00394.
[152] Park SH, Sung JH, Chung N. Berberine induces apoptosis via ROS generation in Panc-1 and MiaPaCa2 pancreatic cell lines. Brazilian J Med Biol Res 2015;48(2):111–119. doi:10.1590/1414-431X201404293.
[151] Chen QQ, Shi JM, Ding Z, Xing X, Zhao X. Berberine inhibits angiogenesis in glio-
Jagetia G.C.: Anticancer activity of berberine J Explor Res Pharmacol 37(2):729–736. doi:10.3892(or.2016.5327.
[137] Zhu Y, Ma N, Li HK, Tian L, Ba YF, Hao B. Berberine induces apoptosis and DNA damage in MG-63 human osteosarcoma cells. Mol Med Rep 2014;10(4):1734–1738. doi:10.3892/mmr.2014.2405.
[136] Mishra R, Nathani S, Varshney R, Sirdar D, Roy P. Berberine reverses epithelial-mesenchymal transition and modulates histone meth-
ylation in osteosarcoma cells. Mol Biol Rep 2020;47(11):8499–8511. doi:10.1007/s13392-020-05892-8.
[135] Peng PL, Hsieh YS, Wang CJ, Hsu JL, Chou FP. Inhibitory effect of ber-
[134] Qi HW, Xin LY, Xu X, Ji XX, Fan LH. Epithelial-to-mesenchymal transi-
[133] Jin F, Xie T, Huang X, Zhao X. Berberine inhibits angiogenesis in glio-
[132] Chen J, Huang X, Tao C, Wang L, Chen Z, Li X, Chen QQ, Shi JM, Ding Z, Xia Q, Zheng TS, Ren YB, Li J, Liu F, Jiang S, Liu J, Chen X, Zhang S, Kalaiarasi A, Anusha C, Sankar R, Rajasekaran S, John Marshal J, Muc-
[131] Chen J, Huang X, Tao C, Wang L, Chen Z, Li X, et al. Berberine chloro-
[130] Chen J, Huang X, Tao C, Wang L, Chen Z, Li X, et al. Berberine chloro-
[129] Chen J, Huang X, Tao C, Wang L, Chen Z, Li X, et al. Berberine chloro-
[128] Chen J, Huang X, Tao C, Wang L, Chen Z, Li X, et al. Berberine chloro-
[127] Chen J, Huang X, Tao C, Wang L, Chen Z, Li X, et al. Berberine chloro-
[126] Chen J, Huang X, Tao C, Wang L, Chen Z, Li X, et al. Berberine chloro-
[125] Chen J, Huang X, Tao C, Wang L, Chen Z, Li X, et al. Berberine chloro-

DOI: 10.14218/JERP.2021.00005 | Volume 00 Issue 00, Month Year
[173] Ho YT, Yang JS, Lu CC, Chiang JH, Li TC, Lin JJ, et al. Berberine inhibits human tongue squamous carcinoma cancer tumor growth in a murine xenograft model. Phytomedicine 2009;16(9):887–890. doi:10.1016/j.phymed.2009.02.015.

[174] Tsang CM, Cheung YC, Lui VYW, Yip YL, Zhang G, Lin VW, et al. Berberine inhibits tumorigenicity and growth of nasopharyngeal carcinoma cells by inhibiting STAT3 activation induced by tumor associated fibroblasts. BMC Cancer 2013;13:619. doi:10.1186/1471-2407-13-619.

[175] Chen YX, Gao QY, Zou TH, Wang BM, Liu S De, Sheng JQ, et al. Berberine versus placebo for the prevention of recurrence of colorectal adenoma: a multicentre, double-blinded, randomised controlled study. Lancet Gastroenterol Hepatol 2020;5(3):267–275. doi:10.1016/S2468-1253(19)30409-1.

[176] Kheir MM, Wang Y, Hua L, Hu J, Li L, Lei F, et al. Acute toxicity of berberine and its correlation with the blood concentration in mice. Food Chem Toxicol 2010;48(4):1105–1110. doi:10.1016/j.fct.2010.01.033.

[177] Haginiwa J, Harada M. Pharmacological studies on crude drugs. V. Comparison of berberine type alkaloid-containing plants on their components and several pharmacological actions (in Japanese). Yakugaku Zasshi 1962;82:726–731.

[178] Jahnke GD, Price CJ, Marr MC, Myers CB, George JD. Developmental toxicity evaluation of berberine in rats and mice. Birth Defects Res B Dev Reprod Toxicol 2006;77(3):195–206. doi:10.1002/bdrb.20075.

[179] Yin J, Xing H, Ye J. Efficacy of berberine in patients with type 2 diabetes mellitus. Metabolism 2008;57(5):712–717. doi:10.1016/j.metabol.2008.01.013.

[180] Marin-Neto JA, Maciel BC, Secches AL, Gallo Júnior L. Cardiovascular effects of berberine in patients with severe congestive heart failure. Clin Cardiol 1988;11(4):253–260. doi:10.1002/clc.4960110411.

[181] Fung FY, Linn YC. Developing traditional Chinese medicine in the era of evidence-based medicine: current evidences and challenges. Evid Based Complement Alternat Med 2015;2015:425037. doi:10.1155/2015/425037.

[182] Bateman J, Chapman RD, Simpson D. Possible toxicity of herbal remedies. Scott Med J 1998;43(1):7–15. doi:10.1177/003693309804300104.

[183] Kumar SG. RNA targeting by small molecules: Binding of protoberberine, benzophenanthridine and aristolochia alkaloids to various RNA structures. J Biosci 2012;37(3):539–552. doi:10.1007/s12038-012-9217-3.

[184] Zou Z, Tao T, Li H, Zhu X. mTOR signaling pathway and mTOR inhibitors in cancer: Progress and challenges. Cell Biosci 2020;10:31. doi:10.1186/s13578-020-00396-1.