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ANTICANCER EFFECT OF BERBERINE: REGULATION OF DEATH SIGNALING PATHWAYS

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ABSTRACT
Medicinal herbs had been used as an anticancer therapy. In the last decades, many alkaloids extracted from these herbs are characterized by its antitumor activity. Berberine, as the major component of Berberis plant, was exclusively studied over the last decades. Its effectiveness as an anti-inflammatory reagent, anti-depressor, antibacterial, antiviral, anti-diabetic and more had put it as a center of research especially as an anti-cancer reagent. This inhibitory effect of Berberine was elucidated by the anti-proliferative effects on different type of cancer as lung, breast, colorectal, Bladder, Glioblastoma, and leukemia by regulating different signaling pathways like PI_3K/AKT, JAK/STAT, NF-κB/COX-2, Map-kinase pathway, the accumulation of ROS in the mitochondria as a result of the oxidative stress and other pathways are able to active the apoptotic molecules and senescence pathway. Recently the Autophagy induced cell death by berberine was also demonstrated as a third type of programmed cell death playing a special inhibitory effect on tumors. Here in our review we are going to resume the most recent work on Berberine by highlighting and discussing this activity.

KEYWORDS: berberine, cancer, signaling pathways, autophagy, apoptosis, senescenc.

INTRODUCTION
Medicinal herbs are constituted of multiple compounds with a therapeutic effect used to prevent many diseases since centuries. The medical effect is revealed by many primary and secondary metabolites derived from different parts of the plant (Fruit, root, leaves). The primary metabolites could be: sugars, amino acids, nucleotides, fatty acids and other lipids, on the other hand the secondary metabolites include phenolic compounds like flavonoids and nitrogen compounds for example the steroids.[1] The traditional medicine is used until now by different people basing on the low cost comparing to the allopathic therapy and the resistance to different antibiotics used against the pathogens.[2] The industry suggests a systematic study of these plants to extract metabolites which will be used in the chemotherapy as medicaments to help the conventional therapy and to reduce its side effects.[3] Looking to the developed countries, the medicaments treating the minor affections are derived from medical plants. Actually, several countries used 1500 species of medical herbs: Albania, Croatia, Turkey and Bulgaria. The therapeutic extracts from the important eight plants (*Panax ginseng, l'astragale membranaceus, Rheumspp, Coptischinensis /* 70 *Berberisspp, Allium spp, Paeonialactiflora, Salviamliltiorrhiza, le Ginkgo biloba,*et *le Camélia sinensis)* are commercialized under many galenic forms.[4]

BERBERICIDACEAE
The Berbericidaceae is a family of medicinal plants constituted of 500 species. They are shrub distributed in the tropical region of Asia, Europe and America. A few number of the wild species grow in Himalaya with a height of 1000-2000m, the most frequent species: *B. asiatica, B. aristata, B. chitria, B. osmastonii, B. insignis, B. vulgaris, B. wallichinana, B. coriaria, B. floribunda, B. himalaica, B. lambertii, B. tinctoria, B. virescens, B. nepalensis, et B. petiolaris ombelle*.[5] Several species are grown in gardens for their ornamental leaves and berries. Flowers bloom in the period from February to June attracts bees. The

Assaf1lting honey is a dark color and strong flavor. The bright yellow wood of various species is used to make frames, sculptures and wooden toys.[5] The roots and stems produce yellow dye useful for dyeing leather and fabrics. The root extracts of various species of Berberis are used as a folk remedy in the world for various inflammatory ailments, including rheumatism. Berries are edible, laxative and useful for fever.[6]

BIOACTIVE MOLECULES OF THE PLANT BERBERIS

A number of alkaloids have been extracted from various species.[7] It includes berberine, berbamine, jatrorrhizine, palmitine and isotetrandrine.[8-9] alkaloids as oxyacanthine, berbebamine, columbine, jatrorhizine, isotetrandine, palmatine and berberine are found in aemulans B., B. dasystachya, B.poirettiet B. pruinosa.[10] The alkaloid extract of Berberis thunbergi has antimicrobial activity: it inhibits the activity of Bacillus thuringiensis, Bacillus subtilis and Staphylococcus aureus.[11] The extract Berberis lycium root is used against many infections, including urinary tract infections. In Pakistan, it is used to treat liver diseases.[12] The root extract Berberis aristata has a hypoglycemic effect and this by reducing the activity of glucose 6-phosphatase and increasing the activity of glucokinase.[13] It is known that hyperglycemia can inactivating antioxidant enzymes and cause oxidative stress, this inactivation is decreased in diabetic animals injected with this excerpt: decreased catalase activity and superoxyde dismutase.[13] A preliminary study of the anticancer activity showed that the ethanol extract reduced the tumor volume in mice bearing the Ehrlich carcinoma.[14] The antibacterial activity was found in the berberis aetnensis, it might due to the presence of flavonolignane 5'-MHC-D which inhibits the drug exclusion pump implicated in the resistance to drugs.[15] An anti-inflammatory effect had been observed as a conclusion to the inhibition of COX-2 and PGE_2 produced by the ischemic animals.[16] The extract of roots leaves and strands of Berberis croatica have an antioxidant activity. This is proved by the elimination of the free radicals.[17] A recent study showed that the ethanolic extract specie of Berberis libanotica possess an anticancer effect by targeting NF-κB/COX-2, PI3K/Akt and mitochondrial/caspase signaling to induce human erythroleukemia cell apoptosis.[18]

BERBERINE: EXTRACTED MOLECULE FROM BERBERIS PLANT

The extracts of Berberis plant have numerous therapeutic effects; they can be used to treat a multiple number of diseases such as diabetes, cancer, gastric diseases and inflammatory diseases.[14-18-19-20] While the importance of these medicinal plants is due to the presence of active molecules in their composition, the introduction of this plant in the medical profession imposes a need to search for bioactive compounds. For this purpose, phytochemistry was performed and a large number of compounds have been identified. Berberine (5,6-dihydro-9,10-dimethoxybenzo [g] -1,3-benzodioxole-5,6-aquinolizum) is a natural isoquinoline alkaloid (figure1)with an intense yellow color and a bitter taste (Figure 1). It is found in many medicinal plants and is used in traditional Indian and Chinese medicine. As it is strongly colored in yellow, it is also used as a natural dye. In recent decades, berberine has shown increasing interest in its important biological activities including antioxidant, anti-microbial and anti-cancer.[21-22-23-24-25]

SOURCE OF BERBERINE

The Coptidis rhizome and berberis plants are the main natural sources of berberine. Coptidis The rhizome (called Huanglian) is a popular herb used in traditional Chinese medicine for centuries to treat infections.[26] Its yellow roots contain a high content of berberine. Berberisaristata, Berberisaquifolium, Berberisasiatica, Berberiscroatica, Berberisthunbergi and Berberisvulgaris, are shrubs that grow mainly in Asia and Europe, particularly in India and Iran. Their roots, bark, leaves and fruits are often used in folk medicine.[27]

BIOACTIVITY OF BERBERINE

Berberine has been reported to have several biological activities.[22-28] These beneficial effects could be useful for the prevention and treatment of many diseases like cancer, cardiovascular diseases, diabetes, neurodegenerative diseases.[21-29-30-31-32] The next part mainly summarizes the research developed on its main anticancer activities and potential mechanisms.

ANTICANCER EFFECTS OF BERBERINE

1-Induction of apoptosis

It was found that berberine could induce cell death in various cancer cells such as breast cancer, liver cancer and lung cancer. Apoptosis is the cause of this cell death in many cell lines and xenografts.[33] It has been shown that berberine induces PARP cleavage, nuclear condensation and induces apoptosis rate on FaDu cells.[34] Berberine can activate the mitochondria and caspase dependent apoptotic pathway *in vitro*.[35] Mitochondria is a key target in many apoptotic events, for this reason the effect of berberine on this organelle was studied. Berberine carries out multiple effects on mitochondria thus by inhibiting mitochondrial Complex I and interaction with the adenine nucleotide translocator.[36] It can cause cancer cell lines in the fall of mitochondrial membrane potential, release of cytochrome c and AIF into the cytosol.[37-38-39] It could also regulate the expression of pro-apoptotique Bax and Bak proteins, and regulate the decreased expression of anti-apoptotic proteins Bcl-2 and Bcl-XL (Chen et al., 2009; Eom et al., 2010; Katiyar et al., 2009; Lin et al, 2006). Finally, a number of caspases such as caspase-3, 4, 7, 8 and 9 may be activated by berberine.[37-40] On the other hand, the way of death receptor can be activated in apoptosis induced by berberine.[41] In human cervical carcinoma cells (HeLa), berberine increases the expression of Fas, FasL, TNF-α and TRAF-1 and then activates caspase-8 and caspase-3.[42]

On the other hand, berberine may regulate caspase-independent cell death by inducing the breakage of DNA strands by topoisomerase inhibition.[43]

Many other molecules related to apoptosis have also been reported to be involved in cell apoptosis induced by berberine. The latter could upregulate the expression of p53 and p27 playing a pro-apoptotic role in cancer cells.[35-42] Berberine may also inhibit oncogenes H-ras and c-fos in the cancer cell line T24 bladder,[40] in the same context Barbering have a synergetic effect with Epirubicin (EPI) on Bladder Cancer cells by enhancing G0/G1 arrest, the cleaved caspase 3 and caspase 9, Bax, P53 and P21 and repressing Bcl2[44] and cMyc.[45] It has been reported that beberine can downregulat the mir-21 and increase apoptosis in the U266 multiple myeloma cancer line**;**[46] berberine has been identified to suppress the activity of RET protooncogene by binding and stabilizing the RET G-quadruplex wich further activated the apoptotic mechanism validated with the increasing activity of caspase-3 in the human medullary thyroid carcinoma cell line (TT) and HEK-293 cell line.[47]

2-Senescence

The antitumor activity of berberine derivatives had been revealed by many studies during the last decades. A study showed that many synthetic derivatives of berberine: NAXO12, NAXO13, NAXO14, NAX035 induced senescence by increasing the expression of senescence markers as p53, p21, p16, PAI-1 and the reduction of HER-2/neu expression and phosphorylation on HER2/neu over expressing SK-BR-3 breast cancer cells.[48] In the other hand, another *in vivo* study using transgenic mouse model which spontaneously develops HER2-positive mammary tumors revealed that the repeated intra- peritoneal injections of Berberine (BBR) and its synthetic derivative known as NAXO14 have delayed the tumor development and reduced its number and mass. The anti-angiogenic activity is induced significantly by NAXO14 than BBR by inhibiting the development of the vascular network and its density and inducing senescence.[49] Glioblastoma cells undergoes cellular senescence after treatment with berberine. These treated cells showed a decrease in EGFR level, a downregulation of RAF-MEK-ERK signaling pathway downstream. Thus leading to conclude that the senescence is mediated by the downregulation of the EGFR-RAF-MEK-ERK pathway and berberine could be used in the treatment of glioblastoma.[50] The uncontrolled proliferation is mainly contributed by the chromosome telomere synthesis of cancer cells. The telomere G-quadruplexe structures in cancer cells are a strong target for drugs. It had been demonstrated that BR8, one of 9-substituted beriberine derivatives, could induce the cell cycle arrest, senescence and DNA damage at the telomeric region by the delocalization of TRF1 and POT1 from telomere accompanied by a rapid telomere uncapping which made BR8 as a very effective anti-cancer drug.[51] Another study showed that administration of BBR into cultures of A549 cells undergoing premature senescence reduced the development of senescent phenotype as revealed by analysis of cell morphometric features, activation of SA-β-gal.[52]

3-Autophagy

Autophagy or self eating is considered as a programmed death in cells. This stress adaptation response of cells may contract with apoptosis defined as self killing or interconnect together. The three types of autophagy in literature are macro-autophagy, micro-Autophagy, chaperone mediated- autophagy. The effect of berberine on cells by using autophagy is contradictory. Many publications showed the effect of berberine as an enhancer alkaloid of autophagy unlike other that demonstrates the opposite effect.[53] According to the literature, the antitumor activity of berberine and its derivatives had been studied by many authors. The berberine induction effect of autophagy had been reported. Wang et al had been shown that the induction of the autophagy by the inhibition of AMPK/mTOR/ULK 1 leading to the inhibition of the glioblastoma multiform growth by impairing its glycolytic capacity.[54] Overcoming the resistance to lapatinib, Berberine activates apoptosis and oxidative stress by inhibiting autophagy, DNA synthesis and colony formation capacity of lapatinib resistant HER2 breast cancer cells.[54] Moreover the synergetic effect was verified by the combination of lapatinib and berberine by increasing ROS production and down regulating of c-Myc/pro-Nrf2 and GSK3 (Glycogen Synthase Kinase 3) which is enhanced by the inhibition of autophagy[55] Furthermore, Guaman et al. reported that many derivatives of this alkaloid repress colon cancer cell proliferation leading to the cell cycle arrest, cell death and autophagy by expressing its typical markers.[56] However, the antitumor activity of berberine in hepatocellular carcinoma, characterized by a poor prognosis, was demonstrated through the activation of apoptosis (caspases 9 and 3) and autophagy by enhancing AMPK, MAP kinase complex by up regulating p38, Beclin-1 and mitigating mTORC1.[39] In the other side, Peng et al. showed that berberine increases the cytotoxicity of the radiation by inducing autophagy in lung cancer cells.[57] The antitumor therapy is characterized by the side effects caused by the used therapeutic molecules. Berberine was shown by Domitrovic et al. by its nephroprotective activity against cis-platin induced renal injury by the inhibition of NF-κB, tumor necrosis factor-cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), p53 and activated caspase-3.[53] Berberine could avoid the cardiac dysfunction after an acute myocardial infarction by enhancing self eating through the inhibition of p38 AMPK and the activation P-AKT, with an increase in LC-3BII and Beclin-1. Furthermore, in the same field, Berberine attenuates the cardial dysfunction and reduces the hypertrophy of the heart by the down regulating mTOR, p38 and ERK1/2 signaling pathways.[57-58] It had been published the importance of the berberine in the

idiopathic pulmonary fibrosis which is a fibro-proliferative lung disorder induced by the bleomycin. By reversing the effect of this molecule, berberine abrogated smad 2/3 but not smad 7 and blocked FAK dependent PI3K/Akt by raising the expression of PTEN leading to the stimulation of autophagy by up regulating Beclin-1 and LC-3BII and the inhibition of mTOR.[59] A previous study by X et al showed the autophagy is induced by the upregulation of GR P78 and the inhibition of the ubiquitination and proteosomal degradation of GRP78 and ATF6.[60]

SIGNALING PATHWAYS

Growth inhibition and cell death induction was proven in cancer cells after treatment with berberine. Berberine regulates the constitutive activation of EGFR in human colon tumor, prostate cancer and glioblastoma.[50-61-62] EGFR-MEK-ERK signaling pathway is downregulated in human glioblastoma cells treated with berberine.[50] Berberine was found to inhibit EGFR through activation of Cbl in colon tumor cells.[62] Furthermore, EGFR was inhibited in berberine treatment of prostate cancer cell lines.[61] It has proven that berberine decreases the phosphorylation levels of EGFR both *in vitro* and *in vivo*. Furthermore, it decreases levels of pERK, pAKT, pNFκB and pSTAT3. This facts may suggest a global inhibition of the EGFR signaling pathway.[32-61] Gain of function of EGFR can lead to the upregulation of PI3K/AKT signaling pathway, and thereby drives cell survival and proliferation.[63-64-65] Antitumor activities in osteosarcoma, chondrosarcoma cells, breast cancer cells, are contributed by the inhibition of the PI3K/AKT signalling pathway. Apoptosis in osteosrcoma is correlated with the downregulation of protein expression of PI3K and P-AKT.[66] In addition, berberine induced inhibition of cell proliferation of chondrosarcoma cells through the regulation of PI3K/Akt pathway.[67] Other data also indicates that berberine promotes to apoptosis in breast cancer cells by downregulating HER2/PI3K/AKT signaling pathway.[68] In melanoma cancer cells, berberine induces phosphorylation of PI3K/AKT and ERK causing an inhibition of melanogenesis.[69] Also, berberine have an effect on this pathway in non-solid cancer. It downregulates the protein of p-AKT in leukemic cells.[18] In mammalian cells, treated with Berberine, the appoptotic effect is induced by the down regulation of P-akt and the downregulation of AMP activated protein kinase phosphorylation in a sustained manner.[70] A recent study revealed that the treatment of HEK293 by berberine extracted from Coptidis chinensis inhibit angiogenesis by repression of Ephrin-B2 leading to the downregulation of phosphorylates VEGR2 and its downstream signaling members (Akt and ERK1/2) and metastasis by the inhibition of metalloprotease 2.[71] This natural alkaloid is also effective in inhibiting the expression of STAT3 that directs gastric cancer cells towards cell death.[72] Constitutive and IL-6 induced STAT3 activation inhibited by berberine in NPC cells, revealed by the reduce of level of phosphorylated STAT-3, leads to apoptosis in this cells.[73] A study done on colon cancer cells was able to identify that BBR may inhibit selectively the Hh signaling pathway activity by targeting the component Smo and results in inhibiting the Hh-dependent cancer growth.[74] It is well known that the enzymes PLA_2 and COX-2 involved in the AA metabolic pathway play a key role in progression of many type of cancers.[75-76] Berberine reduced the expression level of those 2 enzymes in hepatocellular carcinoma, consequently involving in its inhibitory effects.[77] An inhibition of COX-2 expression and PGE_2 levels appears in colon cancer cells treated with berberine.[78] Also, a reduction of COX-2 expression was noticed in leukemic cells treated with berberine. Berberine combined with luminespib (NVP-AUY922)causes growth arrest in cells via inhibition of the kinase Cdk4 and suppressing the pin1-β-catenin-cyclin D1 pathway.[79] Berberine alone inhibits the transcriptional activity of β-catenin, and as a result, active β-catenin was lowered with an increase in the expression level of e-cadherin.[80] Recent research shows that cellular stress-activated AMPK can enhance cell apoptosis.[81] This suggestion is validated also by berberine. It inactivates the mTORC1 and simultaneously activates the AMPK, directing cells to apoptosis and to autophagy.[82] In addition, berberine suppresses tumorigenesis in colon cancer cells via AMPK dependent inhibition of mTOR activity.[83] Berberine extracted from Rhizoma coptidis exhibit potent antitumor activity by the abrogation of NF-kB pathway leading to apoptosis and an antiproliferative effect.[44]

INCREASE SENSIBILITY TO CHEMOTHERAPY AND RADIOTHERAPY Moreover, berberine also showed synergistic anticancer effects in combination treatment of cancer with chemotherapeutic agents or radiotherapy. The encouraging results of studies suggest that berberine might have potential to be developed as an effective adjuvant anticancer agent. However, the hermetic dose response of berberine has not been evaluated yet. This information could be imperative to provide suggestions for potential clinical applications, particularly in the treatment of cancer. Studies suggest that berberine could enhance and increase the efficacy of chemotherapy drugs. Berberine may be a prometant therapeutic drug in gastric cancer, it can enhance the activity of EGFR inhibitors.[32] Berberine combined with cetuximab increases level of cleaved PARP and decreases level of Bcl-XL.[32] The combination of berberine with tamoxifen inhibits cell growth of MCF-7 more powerfly than tamoxifen alone. Berberine sensitizes A549 and Hela cells towards the anticancer effect of doxorubicine, the IC_{50} of combination of berberine and doxorubicine was higher than the IC_{50} of doxorubicine used alone.[84] Berberine refines chemosensitivity to CPT-11in human colorectal cancer HCT116 cells through the activation of the inhibition of NF-κB. Berberine suppressed CPT-11-mediated NF-κB activation in a dose-dependent manner and potentiated apoptosis by the downregulation of NF-κB antiapoptotic

target genes.[85] In recent study, mir-203 expression was induced in gastric cancer cell lines SGC-7901 and BGC-823 after treatment with berberine, and an induce of sensitivity to cisplatine by apoptosis pathway was also observed.[79-86-87] Also berberine may be used as an adjuvant in radiotherapy. It can sensitize prostate cancer cells and esophageal squamous cancer cells to radiotherapy due to its inhibition of the expression of VEGF and HIF-1 factor.[88] In recent years, berberine has been noted as a potential candidate for the treatment of cancer. Its anti-cancer effect has been attributed mainly to its actions in the induction of death, growth suppression and inhibition of metastatic cancer cells.

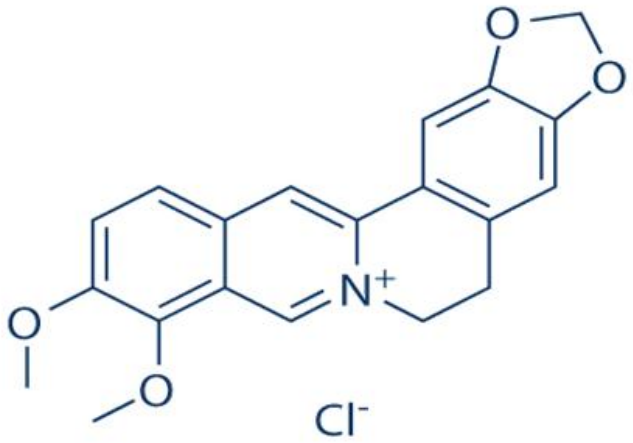

Figure 1. Chemical structure of berberine.

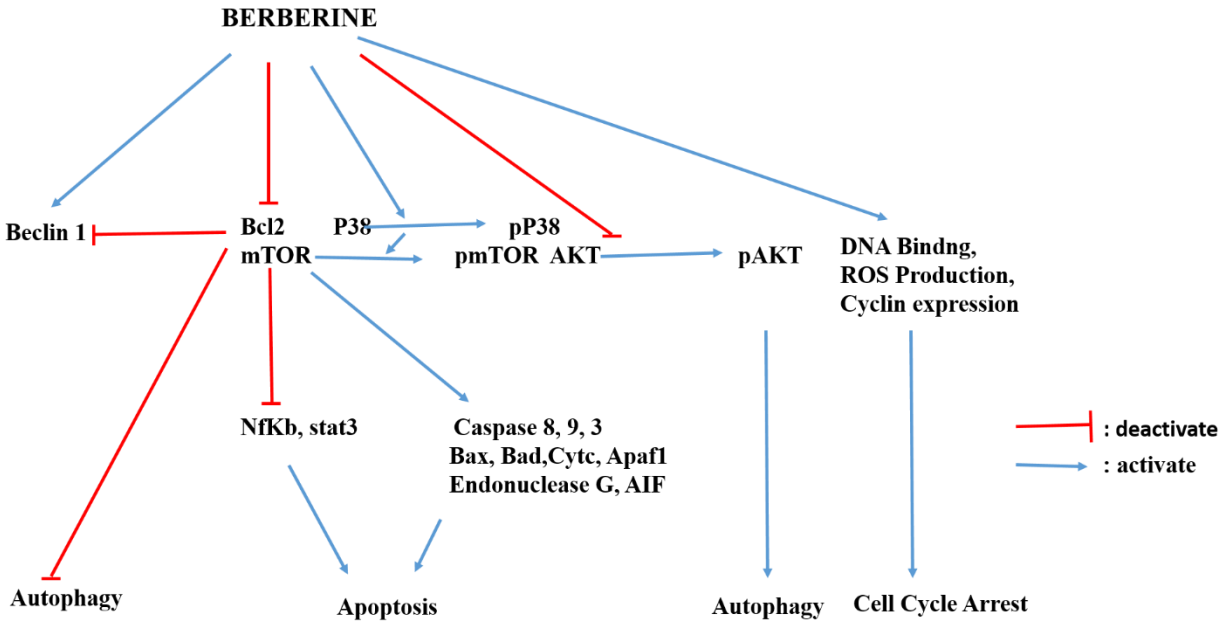

Figure 2: Regulation of signalling pathway by berberine thus leading to cancer cell death.

CONCLUSIONS

In conclusion, this review showed that the natural compound berberine possess a therapeutic effect in cancer. It can exhibit anti-proliferative activity by arresting the cell cycle. It can also induce cancer cell death through activation of apoptosis, senescence and autophagy pathways (Figure 2). The modulation of a multiple number of molecules by berberine, such as: BCL-2, bax,bad, cyclins, p38, pAKT, caspase8,caspase9,caspase3, NFkb and many others implicated in survival pathways, plays a crucial role in targeting cancer cells. The alkaloid berberine is extracted from a variety of plants, so far it could be a prominent molecule used in therapy programs for cancer patients to improve the effectiveness of the treatment

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