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Exploring the role of berberine as anti-cancer agent in cervical cancer

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Abstract
Berberine is one of the natural alkaloids that is extracted from various medicinal plants and possesses multiple bioactivities including antioxidant, anti-inflammatory, anticancer, and antimicrobial. The traditional use of Berberine has received scientific interest due to its possible treatment options in various diseases, most notably in cancers. This review mainly focuses on cervical cancer which is a frequently reported cancer among women, particularly in the developing country, and HPV is a leading cause. Even though HPV vaccines and screening interventions exist, cervical cancer continues to be a global threat that affects women. In present-day treatment, options like surgery, chemo and radiation are normally associated with various adverse impacts on the patient and are not very effective, especially in the advanced stages of the illness. Berberine possesses a promising anticancer effect on cervical cancer cells. Berberine is reported to play several mechanisms towards anticancer effects on cervical cancer cells including apoptosis, cell growth inhibition and inhibiting signalling pathway and it gives promises a plant-based therapeutic option to boost the effectiveness of current treatments.

Keywords: Berberine, natural alkaloid, cervical cancer, HPV, anticancer effect

Introduction
Berberine is a natural alkaloid based on quaternary isoquinoline and a bioactive compound that occurs naturally. Numerous plant families and genera have been found to contain berberine, they are Annonaceae, Berberidaceae, Menispermaceae, Papaveraceae, Ranunculaceae, and Rutaceae. As a natural source, Berberine is widely present in the genus Berberis. It is widely distributed in the different parts of plants like stems, roots, leaves, rhizomes, barks, and twigs [1].

Table 1: Example for source of berberine [1]

Sl. No.	Species	Used parts
1	*B. aristata*	Bark, Roots, Stem, Fruit,
2	*B. aquifolium*	Roots
3	*B. heterophylla*	Root bark
4	*B. chitria*	Whole plant
5	*B. vulgaris*	Stems and roots
6	*C. chinensis*	Root
7	*C. japonica*	Rhizome
8	*Sinopodophyllum hexandrum*	Roots, rhiome
9	*Coptis chinenaia*	Roots
10	*Tinospora cordifolia*	Stem, Leaves, root
11	*Xanthorhiza simplicissima*	Bark of root, trunk and Perennial Branch, Annual braches, leaves.
12	*Zanthoxylum monophyllum*	Stems and branches

Protoberberine alkaloids and derivatives are a class of organic compounds that include berberine. Berberine structure is based on a protoberberine moiety, which is 5, 6-dihydrodibenzene fused with a quinolizinium nucleus to form 5,6-dihydrodibenzo (a,g) quinolizinium skeleton [2]. Berberine has the chemical formula C20H18NO4 and a molecular weight of 336.337g/mol. Berberine has been utilized to treat various diseases in Indian Ayurvedic and traditional Chinese medicinal systems [3, 4]. Berberine exhibits numerous physiological effects including antioxidant, anti-inflammatory, anticancer and antimicrobial against Viruses, Bacteria, Parasites and fungi. Along with it can overcome antibiotic resistance [5, 6].

Berberine is extracted from plant sources by different extraction methods are employed and they are percolation, maceration, soxhlet, and continuous hot and cold extraction procedures are frequently used with different solvents, including methanol, ethanol, chloroform, aqueous solutions, and acid mixtures. Berberine is heat and light-sensitive hence during the extraction process any variation in this condition may lead to the degradation of berberine [7, 8, 9].

Cervical cancer and HPV Infection

The abnormal growth and division of cells in the cervical region is referred to as "cervical cancer." The endocervix, a part of the cervix next to the uterus, is made up of simple columnar epithelial cells. The cervix forms the lowest part of the uterus and has different linings. On the other hand, stratified squamous epithelial cells border the ectocervix, which is next to the vagina [10]. Within the endocervical canal, the squamocolumnar junction is made up of stratified squamous and columnar epithelium. The metaplastic epithelium of the "transformation zone" replaces the columnar-lined epithelium of the endocervix when these regions converge. This region is the most likely to have premalignant transformation from ongoing HPV infection, making it the most likely place for cervical cancer to occur [10, 11]. Based on this histology of the cervix, cervical cancer is classified into three subgroups: adenocarcinoma carcinoma, squamous cell carcinoma and adenosquamous carcinoma. Mucus-producing glandular cells in the endocervix are the source of adenocarcinoma. Approximately 85% of all cases is squamous cell carcinoma and it is the most common of these subtypes [10].

In 2020, there were an anticipated 123,907 new instances of cervical cancer diagnosed in India and 77,348 cervical cancer deaths. Cervical cancer is the fourth most frequent cancer worldwide and the second most common type of cancer among women in India between the ages of 15 and 44. In India, the average incidence of cervical cancer per 100,000 people ranges from 6.5 to 24.2 [12]. Approximately 85% of cases have been identified to be in advanced and terminal stages, and between 63% and 89% of patients had regional illness at the time of diagnosis. Consequently appropriate screening programs and prophylactic vaccinations are required for the early identification and prevention of cervical cancer [10].

The primary cause of cervical cancer is 10-20% prolonged infection with human papilloma virus which is oncogenic [13] and it is also associated with the early onset of sexual intercourse, oral contraception, multiple sexual partners, immunosuppression and smoking [14, 10].

Human Papilloma Virus belongs to the family Papillomaviridae. It has circular non-enveloped double-stranded DNA of diameter 50-55 nm. HPV is classified into five different genera and they are alpha, beta gamma, mu and nu. Again this group consists of different types of HPV. In these different genera Alpha papillomaviruses are common and cause 5% of cancer worldwide [15]. HPV infection types 16 and 18, which have a high risk of cervical cancer, are responsible for about 75% of cases and there are other HPV types also responsible for the development of cancer. In particular type 11 and type 6 are the low-risk HPV types. Even while over 500,000 new cases of HPV are discovered every year, the majority are low-grade infections that go away on their own after two years [16, 17]. The viral oncoproteins E5, E6 and E7 within HPV DNA disrupt the host cell cycle; of these, E5 interact with the growth regulation factors of the host, the platelet-derived growth factor β receptor, epidermal growth factor receptor and the colony stimulation factor 1 receptor [11]. E6 disrupts the suppressive tumor protein p53 and E7 disrupts the retinoblastoma protein (pRB) and both p53 and pRb are involved in the cellular checkpoint [16]. The E5 protein is involved in immunological evasion. E5 also has a role in HPV-related neoplasia. Oxidative stress and certain microRNAs are involved in cervical cancer development [17]. The three diagnostic tests most frequently employed for early detection of cervical cancer are the high-risk HPV-only test, the Pap-only test, and the Pap-HPV co-test [18, 19, 20 and cytology-based testing, HPV-based testing is needed for the screening of cervical cancer [21].

Importance of Berberine as an anticancer agent in Cervical Cancer Treatment

Treatment and management of cervical cancer are determined based on the stage of cancer whether it has spread to other areas of the body, tumor size, the patient's age general health. The treatments for cervical cancer include surgery, radiation, chemotherapy either separately or in combination, and immunotherapy [22, 23, 24]. Diagnosis of cervical cancer and the treatment that follows can result in serious morbidity, which makes more distressed and have a lower quality of life. Since many occurrences of cervical cancer manifest before the age of 49, it is crucial to take treatment's long-term adverse effects into account since they may have an impact on quality of life. These young women might have greater survival rates, but they might also experience long-term side effects from their medication, like infertility, early ovarian failure, and bowel or bladder problems [22]. To overcome this problem there are plant-based medications with anticancer properties and fewer adverse effects should be considered [25]. Phytochemicals were among the few plant-based medications that were used. Berberine, a well-known natural substance, has been demonstrated to be an effective anticancer agent. Berberine exhibits strong anticancer properties. The mechanism of action involved was by causing cell apoptosis, preventing cell invasion and migration, cell cycle arrest, modulating inflammation, transcription factor, genetic pathway and reducing the viability of cervical cancer cells. Along with this its pharmacokinetics, and effectiveness, safety against a variety of disorders have all been studied [26, 27].

Berberine induces Apoptosis via Mitochondrial-mediated Pathways, Death Receptor Signaling, MAPK Pathways, by Up-Regulating p53 and Inhibiting HPV Oncoproteins in Cervical Cancer Cells.

Berberien triggers apoptosis through mitochondrial-mediated pathways, several death receptors, upregulation of tumor suppressor protein p53, and mitogen-activated protein kinase (MAPK) signalling pathway in the cervical cancer cell line. Berberine demonstrated a very high anti-cancer effect on cervical cells with more emphasis on HeLa cells via several paths that lead to apoptosis. There are three major classes, including multidomain anti-apoptotic such as Bcl-2, multidomain pro-apoptotic and BH3-only pro-apoptotic. These differ in terms of Bcl-2 homology (BH domains) and function Moreover, it is divided into multidomain proapoptotic proteins and duplicated BH3 domain only proapoptotic. By downregulating Bcl-2 expression levels, berberine hydrochloride a commercially

available berberine compound, up-regulates p53 expression levels and initiates the intrinsic route of apoptosis in HeLa cells. Berberine causes a downregulation of the anti-apoptotic protein Bcl-2 and upregulation of the pro-apoptotic protein BAX. Such a change in the Bcl-2/Bax ratio results in the liberation of cytochrome c from the mitochondria to the cytosol, which establishes mitochondrial-mediated apoptosis. Cytocrome delay system; cytochrome c is released and binds to Cytocrome that activates CASP -3, and thus apoptosis leads to the dismantling of the cell. Furthermore, berberine was observed to enhance cleavage from caspase-8 to support the programmed cell death [28]. Berberine can cause apoptosis and induce cell cycle arrest in the G0/G1 and G2/M phase [29, 30]. The extrinsic route is impacted by anticancer medication, which targets Cox-2. It is linked to the promotion of angiogenesis, tumor growth, invasion, and metastasis, and its expression rises with the development of malignant cells. Berberine on dose-dependent suppresses the Bcl 2, and cox 2 expression and increases the p53 expression. Also, Few studies mention that berberine blocks the inner inhibitor MDM2 at the post-transcriptional stage, thereby up-regulating the production of p53 [28]. Apoptosis is initiated by the activation of caspase-9 and the release of cytochrome c in the mitochondrial membrane. This brings out the fact that this mitochondrial dysfunction is a fundamental cause of the berberine-induced apoptosis of cervical cancer cell lines. It has been identified that the death receptor pathway is another important mechanism by which berberine exerts its action. Berberine increases the level of death receptors such as Fas, Fas Ligand; TNF-alpha and TNF receptor-associated factor 1. They belong to the extrinsic apoptotic pathway, whereby cell death is controlled by death receptor-ligand interactions encountered from outside the cell membrane. The activation of Fas and FasL and increased caspase-8 activity enhances the signalling that leads to apoptosis. A well-defined member of pro-apoptotic caspases, caspase 8 initiates the extrinsic apoptosis pathway; hence addition to the mitochondrial signals, berberine can provoke cell death through external death stimuli [31]. Berberine also affects the MAPK signalling pathway involved in the control of cell division, maturation, and programmed cell death. The study also revealed that berberine affects the activity of MAPKs in HeLa cells; the increased phosphorylation of ERK and JNK and the reduced phosphorylation of p 38. ERK activation appears to support cell proliferation, yet consistent with the literature, berberine was shown to inhibit ERK activity at later time points and thus inhibit cell proliferation. The activation of another MAPK is constitutively active JNK, and an increase in apoptosis indicates that the JNK is prominent in berberine-mediated cell death. However, it was shown that p38 is usually involved in stress-induced apoptosis and the studies have proven that berberine can inhibit this process and thus has a stronger effect on other MAPKs than it is observed for stress conditions of cells [31].

Berberine up-regulates p53, a protein that is responsible for controlling apoptosis in response to stress. When p53 was upregulated in HeLa cells after berberine treatment, A crucial regulator of apoptosis, p53 triggers the intrinsic pathway by activating the transcription of Bcl-2 family members and causes DNA damage which leads to p53 activation and apoptosis. Targeting p53, berberine has been demonstrated to modify epigenetic changes and to cause disruptions in the microtubule network [32]. Gene transcription of CDK inhibitors like Kip1 (p27) and Cip1 (p21) can be activated by the p53 mRNA level. It has been demonstrated that berberine increases Cip1 (p21) expression by over 15 times [33]. In cervical cancer, the expression was discovered that berberine treatment decreased the amount of (HPV) proteins E6 and E7 which play an important role in inhibiting p53 and pRb. Additionally, berberine could successfully upregulate the expression of p53 and Rb in HPV-positive cervical cancer cell lines, which might be useful in preventing the growth of cervical cancer cells [34].

Bereberine inhibits the Angiogenesis to halt Tumor Growth and Metastasis.

Endothelial cells are responsible for angiogenesis process where which helps in the formation of new blood vessels. Berberine has ability to suppresses the endothelial cells. The tumor needs angiogenesis and it increases in vascular density allows tumor cells to have better blood flow for its growth, survival and metastasis. The study has demonstrated that berberine reduced the formation of endothelial tube networks, which is a sign of angiogenesis, and the invasive capability in an *in vitro* model. It also inhibits the release of VEGF, a strong pro-angiogenetic factor, and has a negative influence on the regulation of HIF-1α, a transcription factor needed for VEGF synthesis when oxygen concentration is low. Less VEGF production means that inhibition of tumor stimulated endothelial cell growth and new blood vessel formation. The results presented in this research also demonstrated that the down-regulation of VEGF was observed in berberine-inhibited SiHa cervical cancer cells in a dose-dependent manner [35].

Berberine has the ability to hinder EMT; it is the process where epithelial cells acquire the ability to transfer Mesenchymal cells reduced the viability of cervical cancer cells, blocked cell migration and invasion, and promoted programmed cell death by suppressing the production of keratin (KRT). Several investigations on keratin 17 (KRT17) cervical cancer research were conducted and it was found that the KRT17 was involved in cancer keratinocyte cell survival migration/invasiveness of cervical cancer. KRT17, a type I keratin protein, is usually overexpressed in cervical cancer and it relates to a poor prognosis. It supports cancer progression through the promotion of cell viability and suppression of apoptosis. In particular, KRT17 up-regulates Bcl-2 protein, down-regulates Bax and cleaved caspase-3, facilitating inhibition of cancer cell apoptosis [36].

Berberine has significant evidence of suppressing the oncogenic impact of KRT17. The study revealed that berberine inhibited cervical cancer proliferation from over-expressing KRT17 and altered the expressions of apoptotic regulator. Berberine suppressed the level of Bcl-2, an anti-apoptotic protein, while the levels of Bax and cleaved caspase-3 protein, which is pro-apoptotic were enhanced. This leads to apoptosis and indicates that berberine operates to overcome KRT17 expression. Other than influencing cell survival and apoptosis, KRT17 is also involved with cell migration and invasion; two important steps involved in the metastatic process in cervical cancer. KRT17 increases and promoting EMT, a mechanism where epithelial cells gains mesenchymal transition, consequently, increased motility and invasiveness. KRT17 overexpression resulted in increased mesenchymal markers, MMP-9, N-cadherin, and vimentin together with decreased epithelial marker E-

cadherin. These changes are nonspecific but indicate EMT, a process strongly linked to cancer metastasis. Nevertheless, it has been reported that berberine can suppress this process and restore the above mentioned markers to decrease the migration and invasion ability of KRT17 high-expressed cervical cancer cells [37]. Berberine also boosted the expression of GADD153 by generating ROS, which in turn caused mitochondrial malfunction, activated caspase-3, and released cytochrome C, all of which contributed to the demise of cervical cancer cells. Additionally, by down-regulating VEGF, berberine may lessen tumor-induced angiogenesis [38].

Table 2: Dosage, effect, and mechanisms of berberine on cervical cancer cell lines from *in vitro* studies [38].

Sl. No.	Study type	Cell line type	Dosages used	Effects and mechanism
1	*In vitro*	HeLa, Siha, and CaSki cells	5, 10, 15, 20 µM	Reverse EMT by blocking N-cadherin and snail-1 and increasing E-cadherin. Downregulate VEGF to reduce angiogenesis.
2	*In vitro*	SiHa cells and Hela cells	3, 10, 30, 100, and 300 µmol/L	Cell proliferation is inhibited Trigger cell apoptosis and induced cell cycle arrest at G1 phase
3	*In vitro*	Ca Ski cells	0, 50, 100, 150 µM	Increased expression of GADD153 through inducing ROS production, reduce mitochondria dysfunction; trigger the release of cytochrome C and caspase-3.
4	*In vitro*	Hela cells	0.098, 0.195, 0.391, 0.781, 1.563, 3.125, 6.25, 12.5, 25, and 50 µM	Glucose metabolism is regulated via the PI3K/HIF- pathway to Overcome the Radio-resistance

Berberine Inhibits Cervical Cancer Progression through AP-1 Mediated Pathways and Telomerase Suppression.

Expression of the HPV oncogene is due to the presence of host transcription factors. Activator protein or AP-1 belongs to the Jun and Fos proteins which contain JunB, c-Jun, JunD and FosB, c-Fos, Fos-1, 2 respectively. A main function for AP-1 in HPV-mediated cervical cancer was shown by the total loss of transcriptional activity of the E6 and E7 promotor that resulted from mutation of the AP-1 sequence inside the binding sites of the HPV upstream regulatory region (URR). This protein can develop malignancies in a variety of tissues. Berberine downregulates the expression of HPV oncogenes by specifically inhibiting constitutively activated AP-1 in a dose-dependent and time-dependent manner [39, 40]. Additionally, AP-1 inhibition was accompanied by modifications to the makeup of their DNA-binding complex. Oncogenic JunD and c-Fos expression was particularly downregulated by berberine, and this protein was not present in the AP-1 binding complex. Berberine inhibit the C-Fos protein expression by inhibiting the ERK1/2 through TCF/Elk-1 transcription factor. Berberine also triggers apoptosis by poly ABP-ribose polymerase [PARP-1) cleavage. Additionally, berberine reduced the expression of the telomerase protein, hTERT, which prevented cervical cancer cells from growing [40].

Berberine's effect on Cervical Cancer cells radioresistance.

Cervical cancer treatment also includes radiation therapy and one of the most significant factors that cause the formation of the metastatic process and recurrence is radioresistance. Tumor radio-resistance is indicated by conditions such as hypoxia and low glucose levels, characteristic of the tumor environment, including the stabilization of hypoxia-inducible factor 1 (HIF-1). HIF-1 promotes tumor survival because it regulates glucose metabolism and adaptation to low-oxygen conditions. The study explained the berberine, anticancer effects, to reverse P-gp mediated resistance through the PI3K/HIF-1 pathway [41]. When HeLa cells were exposed to hypoxic and low glucose settings as opposed to normoxic and high glucose environments, berberine significantly harmed the cells. Pretreatment with low concentrations of berberine reduced the expression of phosphorylated PI3K and HIF-1α and was indicated that berberine alters nutrient-deprived tumour microenvironment through this signalling. Furthermore, the study showed that berberine can improve radiosensitivity in these unfavourable circumstances, which may be useful in therapy. Berberine blocks the phosphorylation of PI3K which leads to up-regulation of the HIF-1α stabilizing molecular that contributes to the survival of cancer cells under hypoxic stresses hence the anticancer activity. The study also found the reduction of cyclin-dependent kinase inhibitor 1B (CDKN1B) through microarray analysis that is a downstream molecule of hypoxia-inducible factor 1(HIF-1) confirming the effect of berberine on cell cycle and radiosensitivity. In addition, by using bioinformatics analysis, we also found out that several differentially expressed genes (DEGs) were related to the HIF-1 signalling pathway, such as VEGFA, CCND1, and CDKN2A. Most of these genes are involved in activities such as the formation of new blood vessels and cell division, events which are very relevant in the advancement of cancer and in the ability of the disease to respond to treatment. In normal circumstances, PI3K phosphorylates AKT and then activates mTOR this later enhances HIF-1α to control protein synthesis, cell proliferation, and survival. The inhibition of phosphorylation of PI3K, AKT, and mTOR by berberine appears to decrease the activity of these pathways and, in turn, decreases the levels of HIF-1α. This results in reduced utilization of glucose with enhanced radio sensitivity of the cervical cancer cells especially under a hypoxic and low glucose environment. However, this study has shown that due to the inhibition by berberine of the PI3K/AKT/mTOR signalling pathway, the survival pathways of the radioresistant cancer cells are rendered increasing their susceptibility to radiation treatment. This indicates that interfering with this signalling, and consequently, its impact on the HIF-1α protein could be a rational approach for treating radioresistance in cervical cancer [42].

Conclusion

Berberine has a wide range of pharmacological properties and it has been utilized to treat various diseases in Indian Ayurvedic and traditional Chinese medicinal systems**.** It has a natural bioactivity source and is safe, well-tolerated and less toxic. It shows promising anticancer properties by affecting various signalling pathways. Other than cervical cancer it also showed the anticancer activity in gastric, lung, liver, breast, prostate, ovarian and gastric cancer. Berberine could enhance chemotherapy effectiveness when used in combination. Berberine is reported to play several mechanisms towards anticancer effects on cervical cancer

cells including apoptosis, cell growth inhibition and inhibiting signalling pathway. By the intrinsic pathway, it leads to the activation of apoptotic proteins such as Bax and inhibition of anti-apoptotic proteins like Bcl-2. Berberine also activates caspase 3 and caspase 9, caspases which promote apoptosis. Further, it inhibits the HPV oncogene and oncogene products E6 and E7 that regain the ability of p53 and pRb to act as tumor suppressor proteins. It also influences MAPK, PI3K/AKT/MTOR signalling molecules leading to apoptosis of cervical cancer cells. Other mechanisms of anti-tumor activity of berberine include its inhibition of angiogenesis, down-regulation of the formation of vascular endothelial growth factor, and prevention tumor growth by limiting available blood vessel formation. Berberine has the ability to reverse the radioresistant in cervical cancer cells. Overall, it is again shown that berberine has a multifunctional impact on cervical cancer cells - from apoptosis induction to overcoming treatment resistance - which makes berberine promising alternative and complementary treatment. The present date on berberine is only based on the *in vitro* and *in vivo* studies. Furthermore, preclinical studies are required to optimize the optimum dosage of berberine and its safety. Berberine has low bioavailability and relatively low solubility which leads to challenges for promising therapeutic use to overcome this, research findings have improved this bioavailability by developing new technology based on nanomaterials. This natural compound-based treatment gives hope for cervical cancer clinical research in its functional applicability so that cancer can finally be managed more effectively and with fewer adverse effects. In the future, studies with berberine on different cancer types should be examined as well as further comparative mechanisms of action.

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