Plumbagin as a mutli-target drug in treatment of cancer: A review

Prashant N. Amale[1], Shilpa A. Deshpande[2]

[1,2]Assistant Professor, Dept. of Pharmacology, Priyadarshini J.L. College of Pharmacy, Nagpur, Maharashtra

***Corresponding Author:**
Email: shilpa18@hotmail.com

Abstract

Cancer is the fastest growing disease and leading cause of death in the world. Several drugs are available for the treatment of cancer with their merits and demerits, but not a single molecule is available which claims to cure cancer completely. Plumbagin (PL) is a napthoquinone derivative which is obtained as secondary metabolite from plumbago plant family, *plumbaginaceae*. Plambago plants are the flowering plants naturally occurring in Asia, *Plumbago zeylanica* specially found in India. Previously it was known as "chitraka" (in Ayurveda) whose root extracts were used for the treatment of dyspepsias, piles and diarrhoea. PL found to arrest the cell proliferation, metastasis and growth in various in-vitro and in-vivo studies Several researchers showed that PL modulates cytokines like interleukins, nuclear factor kappa B (NF-kB), tumour necrosis factor alpha (TNF-α), reactive oxygen species (ROS), phosphatidyl-inositol 3 kinase (PI3K) & p38 mitogen-activated protein kinase (MAPK), DNA degradation, jun n-terminal kinase (JNK), protien kinase-C (PKc), mechanistic target of rapamycin (mTOR) and signal transducers and activators of transcription (STAT) which are key player in the growth and development of normal cell at molecular level. PL found to arrest rapidly dividing cells, due to these features and activities, PL can be used in treatment of different kinds of cancers. This article provides an overview on the anti-cancer mechanism and speciality of a single agent PL, as a multi-target molecule obtained from plumbaginous plant in treatment of various types of cancer.

Keywords: Plumbagin, Multi-target, Anticancer, Apoptosis.

Introduction

Cancer is the uncontrolled growth of cells, which can invade and spread to distant sites of the body. Cancer can have severe health consequences including death. About 8.2 million people die each year only because of cancer, which constitutes 13% of all deaths worldwide (WHO). Cancer is a fastest growing disease and considering the journey of cancer disease around 70% cases will increase in next 2 decades. Cancers of lung, prostate, colorectal, stomach and liver are the most common types of cancer in men, while in females breast, colorectal, lung, uterine, cervical and stomach cancer are common.. Early detection, accurate diagnosis and effective treatment, including pain relief and palliative care help to increase cancer survival rate and reduce sufferings. Treatment options include surgery, chemotherapy and radiotherapy, tailored to tumour stage.

Natural plant products may possess much potential in palliative therapy and supportive strategies of current cancer treatments with lesser cytotoxicity to normal cells compared to conventional chemotherapy. Plant synthesizes variety natural products or secondary metabolites.(1) Out of which naphthoquinones constitute one of the largest and diverse groups of plant secondary metabolites with a broad range of pharmacological activities.(2)

Plumbagin (PL) is a plant naphthoquinone metabolite (5-hydroxy-2-methyl-1,4-naphthoquinone) and an active principle found in *Plumbaginaeace*, *Droseraceae* and *Ebenceae* families. Out of these, *Plumbagenaeace* species contain more amount of PL. *Plumbagenaceae* is a family of ten genera and about 300 families mostly found in semiarid regions of the Mediterranean and central Asia. The compound is generally extracted from the roots of Plumbago species which has been ascribed with remarkable medicinal properties.(1) The oldest reference to this plant is found in the ancient Indian Ayurvedic texts of Charaka (second century B.C.) where the plant was known as ''Chitraka,'' a perennial herb, whose roots were credited with therapeutic properties against skin disease, abdomen enlargement, anaemia, diabetes, leprosy, dyspepsia, elephantiasis, diarrhoea and leprosy.(3,4)

PL is notable for its high therapeutic efficiency and minimal side effects, A quinone core is the functional group of PL, which can render a variety of pharmacological activities hence known as pleotropic drug.(1) Based on the some in vitro and in vivo studies, PL can lead to cell cycle arrest via its interaction with cell cycle checkpoints. PL can also induce cancer cell apoptosis and autophagy by inhibition of nuclear factor-κB (NF-κB) activation,(5) up-regulation of p53 via c-Jun N-terminal kinase (JNK) phosphorylation(6,7) and inhibition of phosphatidyl-inositol 3 kinase (PI3K)/protein kinase B (Ak)/mTOR (Mechanistic target of rapamycin) pathway,(8-10) In addition, PL can facilitate the generation of reactive oxygen species (ROS)(11) which consequently leads to the killing of cancer cells. Although PL has shown potent effects in pre-clinical studies,(12) the underlying mechanism is not fully understood.(13)

This review focuses on the possible anticancer mechanism of PL in various types of cancer.

1. Brain tumour: Brain tumour is the most dangerous and life-threatening cancer which may or may not be

curative and the success rate of therapy is less. Glioblastoma multiforme (GBM) is the most common primary brain tumor and is also the most deadly glioma.[14] PL inhibits telomerase in brain tumour cells and results in telomere shortening following chronic long-term treatment. PL treatment resulted in the induction of DNA damage, cell cycle arrest and apoptosis, followed by suppression of the colony forming ability of the brain tumour cells. These effects were substantiated by up-regulation of PTEN (Phosphatase and tensin homolog), TNFRSF1A (Tumour necrosis factor receptor superfamily-1A) and down-regulation of *E2F1* genes along with a drop in MDM2 (Mouse double minute-2), cyclin B1 surviving and BCL2 (B-cell lymphoma-2) protein expression. PL elevated the levels of caspase-3/7 activity as well. This reveals that PL may prove as a potential chemotherapeutic phytochemical in brain tumour treatment modalities.[15]

2. Leukemia: There is wealth of evidence to suggest that ROS can selectively and efficiently modify proteins, thereby regulating cellular signalling. PL can effectively generate ROS that inhibit the activity of topoisomerase-II, which is achieved through stabilization of topoisomerase-II-DNA cleavable complex in HL-60 cells. Reduced topoisomerase-II activity is linked with reduced level of DNA damage. Pre-treatment of cells with NAC (N-acetylcysteine) attenuated PL-induced DNA damage suggesting an essential role of ROS. PL and Shikonin were also shown to induce mammalian topoisomerase II-mediated DNA cleavage in vitro.[11,16] This suggested that formation of a cleavable complex was an essential step in topoisomerase II-mediated DNA cleavage induced by these naphthoquinones. Lawsone and lapacol, which are plant constituents structurally related to PL, could not induce such DNA cleavage. Furthermore, it was determined by a DNA unwinding assay using T4 DNA ligase, where shikonin, lawson and lapacol do not intercalate into DNA, but PL and 2-methyl-1,4-naphthoquinone could do, to a relatively lesser extent than 40 -(9-acridinylamino) methanesulfon-m-anisidide. Involvement of ROS as a mechanism of anticancer activity has been reported in case of human promyelocytic leukemia cells (NB4),[17] on the basis of generation of ROS in PL-induced apoptosis, which was abrogated completely by antioxidant, NAC.[18] The results were confirmed through in vivo studies, using NB4 tumour xenograft in NOD (Non-obese diabetic)/SCID (Severe combined immunodeficiency) mice. The histopathologic examination of tumours and organs showed that intra-peritoneal injection of PL (2 mg/kg of body weight) daily for 3 weeks resulted in 64.49% reduction of tumour volume compared with the control, indicating a possible therapeutic role of PL against myeloid leukaemia. Cell cycle analysis showed that NB4 cells were blocked in G_2/M phase of cell cycle. PL also induced annexin V1/PI cell increase and DNA fragmentation. It was concluded that PL can inhibit cell proliferation, block cell cycle and induce apoptosis of APL cell line NB4 cells.[19] PL treatment was found to cause S-G_2/M phase arrest and induce cell death in MEF cells, irrespective of DNA polymerase status.[20]

3. Acute lymphoblastic leukemia: T-cell acute lymphoblastic leukemia (T-ALL) is one of the most commonly diagnosed diseases. Despite the development of new therapeutic agents, the treatment options for this cancer remain limited. Using the human T-ALL MOLT-4 cell line PL induces a caspase-dependent apoptosis of MOLT-4 cells, with no significant cytotoxicity seen for normal peripheral blood mononuclear cells (PBMCs). Anti-proliferative effects of PL mediated by the activation of mitogen-activated protein kinase (MAPK) pathways, and inhibition of NF-κB (Nuclear Factor k-B) signalling; PL also inhibited LPS-induced phosphorylation of p65 and the transcription of NF-κB target genes. PL is a potent inhibitor of the NF-κB signalling pathway and suppressor of T-ALL cell proliferation and acts as an anticancer agent.[21]

4. Myeloma: The activation of signal transducers and activators of transcription-3 (STAT-3) is well known to positively influence carcinogenesis and therefore, suppression of STAT-3 activation can potentially be crucial to check the growth of cancer cells in myeloma. Researcher found that PL inhibited both constitutive and interleukin (IL)-6-inducible STAT-3 phosphorylation.[22] In an in-vitro study human multiple myeloma cell lines U266 and MM.1S were used to show the beneficial effect of PL. Two natural naphthoquinones, dioncoquinone A and dioncoquinone B, isolated from *Triphyophyllum peltatum*, were found to be active against myeloma cell lines. These results suggest that PL might have a cell-line specific effect in myeloma models.[23] Further detailed studies need to be designed to fully understand the anticancer effect of PL against multiple myeloma.

5. Breast cancer: Breast cancer is a most devastating disease affecting several females in the world. PL exhibited a significant anti-proliferative activity against human breast cancer cells by up-regulation of p53 and p21 and suppression of G_1 cell cycle regulators. Flow cytometric analysis revealed that, PL caused cell cycle arrest at G1 phase which was well correlated with the inhibition of cyclin D1, cyclin E and up-regulation of tumour suppressor protein p53. It further inhibited the expression of anti-apoptotic BCL-2 (B-cell lymphoma) family members such as BCL-xL and BCL-2 and activated pro-apoptotic proteins like Bax and Bak.[24]

PL found to suppress the telomerase activity in cancer cells accompanied by telomere attrition. Telomere shortening was corroborated by reduced telomere fluorescence on chromosome ends and genome instability.[25] BRCA (breast cancer type 1 susceptibility protein) is associated with a large number of cancer-related deaths in women. Triple negative BRCAs, which are characterized by the absence of traditional-estrogen receptor (ER), progesterone receptor and Her-

2/neu(ErbB2) represent a very aggressive subtype of BRCAs. Such BRCAs are usually very difficult to manage clinically. PL significantly inhibited growth of BRCA cells in ER-positive MCF-7 (Michigan Cancer Foundation-7) as well as ER-negative MDA-MB-231 (triple negative) BRCA cells, with no effect on MCF-10A cells, the ''normal'' breast epithelial cells. It was also found to efficiently induce apoptosis in BRCA cells with concomitant down regulation of BCL-2 expression and NF-kB activity. Also, ectopic expression of BCL-2 lead to attenuation of PL-induced effects, which indicated that the anticancer activity of PL against human BRCA cells, including inhibition of cell growth and apoptosis induction is due to inhibition of NF-kB/BCL-2 pathway.[26] PL was shown to exhibit its anticancer activity via autophagic cell death by inhibition of protein kinase B.[27]

6. Ovarian cancer: Several studies were carried to find the effective therapeutic agent for ovarian cancer.

PL can induce selective cytotoxicity to BRCA1 defective ovarian cancer cells. However, the effect of this molecule in BRCA1 mutated breast cancers has not been analyzed yet. It has been reported that ROS induced by PL resulted in DNA DSB (Double strand binding) and activates downstream signalling by ATR (ataxia telangiectasia and Rad3-related protein (Serine-threonine kinase)/ATM kinases and subsequent apoptosis. PL reduces DNA- dependent protein kinase (DNA-PK) expression and inhibits NHEJ (Non Homologous End Joining) activity in BRCA1 defective breast cancer cells. Also, PL induces apoptosis in two different BRCA1 conditional knock out murine models: MMTV-Cre; BRCA1 (Co/Co) and WAP-Cre; BRCA1 (Co/Co), at 2 mg/kg body weight, but 32 mg/kg of carboplatin (CN) was needed to induce apoptosis in them. The apoptosis caused by PL in HR (Homologous Recombination) defective triple negative BRCA1 mutant cell lines and in mouse models occur by inducing ROS mediated DNA DSB. The toxicity profile as compared with Cynide in transgenic mice provides evidence for PL's safer disposition as a therapeutic lead in breast cancer drug development.[28]

Srinivas et al. have analyzed the effect of tamoxifen, emodin, and PL in BRCA1-silenced ovarian cancer cells that express ER. Apoptosis induction was greater in BRCA1-blocked cells, the efficacy being in the order of PL, tamoxifen, emodin. The dose of PL required to kill 50% of cells was 5 mM in the control cells and 2.68 mM for the BRCA1-blocked cells, indicating that the latter was about two-fold more sensitive to PL treatment.[29] In the similar study by Thasni et al. the difference in the ability of some selected compounds to inhibit cell growth in relation to BRCA1 (early onset) status in ER-positive ovarian cancer cells was confirmed. Among the various compounds tested, PL was observed to be the most effective inducer of apoptosis through its binding and modulation of ER-a in BRCA1-silenced cells. Silencing of ER-a resulted in lowering the PL-induced cytotoxicity.[30] These reports suggested chemotherapeutic potential of PL against BRCA1-mutated/defective ER-positive cancers.

7. Cervical cancer: Kuo et al. first demonstrated the anticancer effect of PL against cervical carcinoma HeLa cell line.[27] PL treatment against human cervical cancer cell line ME-18 resulted in induction of apoptosis as evidenced by activation of caspase-3, caspase-9, translocation of phosphatidyl serine, nuclear condensation and DNA fragmentation.[29] PL and its quinone derivatives were shown to be cytotoxic against HeLa cells. These compounds induced changes in morphology, such as blebbing and nuclear condensation, which are indicative of apoptosis. Apoptosis-inducing effect of PL in C33A cells have also been reported which, in combination with low doses of radiation, potently inhibits the cell growth.[31,32] It was also established that PL is capable of suppressing constitutive NF-kB in various cancer cells, which ultimately leads to suppression of downstream NF-kB-regulated gene products.[33] These observations may explain cell growth modulatory, anti-carcinogenic and radio-sensitizing effects of PL. Treatment of BRCA cells with increasing concentrations of PL resulted in a dose–dependent inactivation of endogenous NF-kB in ER-negative MDA-MB-231 cells as well as in the ER-positive MCF-7 cells thus providing a mechanism in support of the killing of both receptor-positive as well as receptor-negative cells.[26] Furthermore, it was established that apoptosis is due to inactivation of NF-kB/BCL-2 pathway. Inhibition of NF-kB/BCL-2 pathway by PL might be an activity that has clinical relevance, particularly for the therapy of advanced and refractory BRCAs.[34] The risk of damaging normal tissue that is normally associated with radiotherapy has hampered its efficacy and the total dose administered. PL Plays a potential role as a radio sensitizer.[35] PL in combination with radiation is able to augment cell growth inhibition very effectively as compared to a higher dose of radiation alone. This combination also found to result in a five-fold increase in caspase-3 activation in C33A cells and BCL-2, Bax and survivin. A bone marrow study of the adult Swiss mouse revealed that, PL exhibit similar activity as cyclophosphamide (CP) with significantly less toxicity. Bone marrow stem cell survival, as evaluated using exogenous spleen colony unit assay, revealed that PL had almost similar inhibitory effect. Collectively, these two studies suggest that PL-mediated radio sensitization is not cancer specific and need to be evaluated in detail for the benefit of patients undergoing radiation therapy. Effect of PL and Cobalt-60 gamma radiation has been studied on Ehrlich ascites carcinoma in vivo, taking cytogenetic damage and cell cycle changes as experimental endpoints. PL-administered (5 mg/kg) intra-peritoneally produced a significant increase in the percentage of S-phase as well as G_2-M cells with a corresponding decrease in the G_1 phase at different post-treatment times. Radiation (7.5 Gy, RT) alone

produced the classical G_2 block at 1 hr. The combination treatment produced the effect as that of RT on G_2-M cells, but its effect on the G_1 phase was more pronounced than the latter. While PL treatments produced a small increase in the percentage of labelled S-phase cells, combination treatment significantly reduced the labelled S-phase cells with a corresponding increase in the unlabelled fraction. Drug or radiation alone significantly increased micronuclei induction at various post-treatment times and the combination of the two further enhanced this effect additively. The mechanism of interaction of PL with radiation in bringing about this effect is not clear.[36] Mouse melanoma cells, when treated with PL at 0.5mg/ml concentration for 60 min either alone or followed by 2 Gy gamma radiation, produced a significant decrease in the cell count on days 3 and 4, whereas radiation treatment significantly enhanced the growth inhibitory effect. These findings further underline the PL-induced radio-sensitizing effects in cancer cells.[37]

8. Prostate cancer: Prostate cancer is another major cancer and second leading cause of cancer-related deaths in men. Cancer stem cells (CSCs) are thought to be responsible for cancer chemo-resistance and relapse, thus they represent a significant concern for cancer prognosis and therapy. Cell proliferation studies showed that both BRCA1/2 siRNA transfected PC-3 (Prostate cancer-3) and DU145 cells exhibited increased sensitivity to PL. Using flow cytometric analysis it was proved that PL has the putative ability to directly target CSCs. Anti-tumour mechanism of PL holds promise for novel therapeutic approaches against BRCA mutated PC-3 cancers as well as CSCs.[38]

Hormone-refractory prostate cancer is particularly very aggressive, difficult to treat and accounts for the majority of prostate cancer deaths. PL can inhibit invasion of prostate cancer cells.[39] In BRCA cells PL was observed to induce apoptosis only in cancer cells but non tumourigenic prostate epithelial RWPE-1 cells were not affected. Additionally, PL (2 mg/kg) delayed tumour growth of implanted DU145 cells in mice by 3 weeks and also reduced tumour weight by 90%. Interestingly, even withdrawal of PL did not result in progression of tumour growth for 4 weeks. This study clearly established in vitro as well as in vivo activity of PL against prostate cancer and suggested the potential role of this compound against hormone-refractory prostate cancer cells. Exposure to PL could significantly reduce the viability of multiple prostate cancer cells with varying receptor status.[40]

9. Pancreatic cancer: Pancreatic cancer is the 12th most common and the most aggressive cancer in the world having the worst prognosis in terms of survival, the incidence and death rates rise with advancing age. The compound inhibited growth of pancreatic cell-1 (PANC-1) and BxPC-3 pancreatic cancer cells in a dose-dependent manner. PL exhibited potent inducing effects on cell cycle arrest in PANC-1 and BxPC-3 cells via the modulation of cell cycle regulators including CDK1/CDC2, cyclin B1, cyclin D1, p21 Waf1/Cip1, p27 Kip1, and p53. PL treatment has shown concentration and time dependent increase in the percentage of autophagic cells and significant increase in the expression level of phosphatase and tensin homolog, beclin 1 and the ratio of LC3-II over LC3-I in both PANC-1 and BxPC-3 cells.[41] Staining and transmission electron microscopic studies have demonstrated morphological changes resembling apoptosis in PANC-1cells treated with PL. The degree of apoptosis was assessed by multiple techniques and significant increment in apoptotic cells was observed. Exposure to PL caused up-regulation of Bax, a rapid decline in mitochondrial transmembrane potential, apoptosis-inducing factor over-expression in cytosol, and cleavage of pro-caspase-9 and PARP (poly ADP-ribose polymerase). Activation of caspase-3, but not caspase-8, was evidenced by fluorometric substrate assay. Pre-treatment with caspase inhibitors did not block PL-induced apoptosis. In an orthotopic pancreatic tumour model, PL markedly inhibited the growth of PANC-1 xenografts without any significant effect on leukocyte counts or body weight. Thus, PL can be potentially developed as a novel therapeutic agent against pancreatic cancer.[42]

10. Liver cancer: The effect of PL on liver cancer $HepG_2$ cells has been investigated and it was observed that PL could inhibit the migration and invasion of liver cancer cells through down-regulation of MMP-2 and uPA. p300 lysine acetyltransferase is closely associated with regulation of many genes and its dysfunction is linked with many disorders. PL has been shown to inhibit histone acetyltransferase activity and p300-mediated acetylation of p53 in vivo.[43] Detailed characterization of interactions of PL and p300 revealed that hydroxyl group of PL forms a hydrogen bond with the p300 lysine residue. Gluconeogenic enzyme levels were elevated in PL-treated animals, indicating the molecular basis of different biological behaviours of 3MeDAB-induced hepatoma and anticarcinogenic property of PL against hepatoma[44] Administration of PL (4 mg/kg) was found to significantly induce regression of tumour in 3-methyl-4-dimethyl aminoazobenzene (3MeDAB)-induced hepatoma in Wistar male rats.[45] In an earlier study, PL was found to be cytotoxic against HEPA-3B hepatoma cell line.[27]

Epidermal growth factor (EGF) and its signalling molecules, EGF receptor (EGFR) and signal transducer and activator of transcription factor 3 (STAT3), have been considered to play a role in liver fibrosis and cirrhosis. PL significantly attenuated liver injury and fibrosis in CCl4 (Carbon tetrachloride) treated rats. At concentrations of 2 to 6 μM, PL did not induce significant cytotoxicity of HSC-T6 cells. Moreover, PL reduced phosphorylation of EGFR and STAT3 in both fibrotic liver and heparin-binding EGF-like growth factor (HB-EGF) treated HSC-T6 cells. Furthermore, PL

reduced the expression of α-SMA, EGFR, and STAT3 in both fibrotic liver and HB-EGF treated HSC-T6 cells. Thus, PL could ameliorate the development of hepatic fibrosis through its down-regulation of EGFR and STAT3 in the liver, especially in hepatic stellate cells.[46] PL significantly decreased $HepG_2$ cell viability in a dose dependent manner. Additionally, treatment with PL significantly increased the Bax/BCL-2 ratio and caspase-3/7 activity.[47]

11. Lung cancer: Prevailing treatment options for lung cancers have limited therapeutic success. This is particularly true for non-small cell lung cancer (NSCLC), which becomes resistant to conventional therapy.

PL was found to show anticancer effects against H460 and A549 NSCLC cancer cells and found that, PL significantly inhibited the growth of H460 cells as compared to A549 cells[48] PL was found to down regulate signalling through EGFR in these cells. Additionally, it effectively caused cell cycle arrest and apoptosis through activation of caspase-3. PL has also been reported to inhibit cell growth through cell cycle arrest and induction of apoptosis in A549 cells.[7,30] PL was found to modulate expression of multiple factors that regulate cell cycle, such as p21, cyclinB1, Cdc2, Cdc25C, etc. Furthermore, PL treatment was found to activate mitochondrial apoptotic pathway and this anticancer action of PL was also verified in a nude mice model. Acharya et al. evaluated the effects of PL on cellular microtubules and PL was found to dose dependently perturb interphase microtubule network and it efficiently inhibited the polymerization of purified tubulin.[49] Recognition of colchicine binding site of tubulin might be responsible for the observed effects of PL. It has the ability to inhibit 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced invasion and migration of A549 cancer cells and this has been attributed to its ability to inhibit the expression of MMPs and urokinase-type plasminogen activator (uPA).[50] Collectively, these studies indicate potential use of PL as an anticancer agent against lung cancer.

PL has a potent pro-apoptotic and pro-autophagic effect on A549 and H23 cells. PL arrests cells in G2/M phase and increases the intracellular level of ROS in both cell lines. PL dose-dependently induces autophagy through inhibition of PI3K/Akt/mTOR pathway as indicated by reduced phosphorylation of Akt and mTOR.[11] Inhibition or induction of autophagy enhances PL-induced apoptosis. There is crosstalk between PL-induced apoptosis and autophagy. These findings indicate that PL initiates both apoptosis and autophagy in NSCLC cells through coordinated pathways[51]

12. Renal cancer: Human embryonic kidney 293 (HEK293) and brain tumour LN229 cells express mainly a renal NAD(P)H oxidase (Nox-4). PL was found to inhibit Nox-4 activity (time and dose-dependently) in HEK293 and LN229 cell examined by chemi-luminescence assay. Production of superoxide in HEK293 cells was inhibited by diphenylene iodonium, a NADPH oxidase inhibitor. The superoxide production in HEK293 cells was NADPH and NADH-dependent indicating that the superoxide was generated by a NADPH oxidase in HEK293 cells, but not by the redox cycling of lucigenin. Furthermore, PL inhibited the superoxide production in Nox-4 transfected COS-7 cells. These results indicate that PL directly interacted with Nox-4 and inhibited its activity.[52]

MTT experiment with PL on RCC (Renal Cancer cell) 786.0 revealed that a 10μM dose of PL is toxic, whereas 1μM- 5μM concentrations can be a range of suboptimal doses for further experiments. The Sub-G_1 analysis with PL treatment confirms that PL indeed behaves as a chemotherapeutic agent and causes cell death via apoptosis. Western blotting shows that PL down-regulates the NF-κB pathway.[33,53] as evident by the time-dependent decrease in the protein products of the pathway. The live/dead assay as well as Sub-G_1 assay with PL or paclitaxel or a combination of both produced results showing synergy between the two drugs caused by apoptotic cell death. The DNA-binding assay further proves the synergy, as well as down-regulation of the NF-κB pathway by PL, which is up-regulated by paclitaxel. These results suggest that PL may potentially be used to sensitize RCC cells to the paclitaxel, as it successfully suppresses NF-κB pathway and its gene products that cause chemo-resistance.[54]

13. Colon Cancer: Activation of AMP-activated protein kinase (AMPK) mediates PL-induced apoptosis and growth inhibition in primary cultured human colon cancer cells and cell lines. Knocking-down of AMPKα by the target shRNA and forced activation of AMPK by introducing a constitutively active AMPK (CA-AMPK) or by the AMPK activator significantly inhibits PL-induced cytotoxicity in cultured and HT-29 colon cancer cell. Exogenously-added short-chain ceramide (C6) enhances PL-induced AMPK activation and facilitates cell apoptosis and growth inhibition. This suggests that AMPK might be the key mediator of anti-tumour activity of PL.[11,55]

14. Oral cancer: Oral and pharyngeal cancer is the eighth most common cancer world-wide. Globally, among these cancers, tongue cancer and oral squamous cell carcinoma (OSCC) are the most prevalent histo-pathological type and exhibits aggressive behaviour. PL dose-dependently suppressed OSCC cell growth, with IC50 values ranging from 3.87 to 14.6 μM. Flow cytometry analysis revealed that PL treatment resulted in a significant decrease in mitochondrial membrane potential and an increase in the number of apoptotic cells. Notably, ROS generation was significantly elevated after PL treatment. Furthermore, NAC, a ROS scavenger, clearly suppressed the decrease in mitochondrial membrane potential, increase in caspase-3/7 activity and apoptosis after PL treatment.[56]

PL induced intracellular ROS generation and this effect was attenuated by l-glutathione (GSH) and NAC.

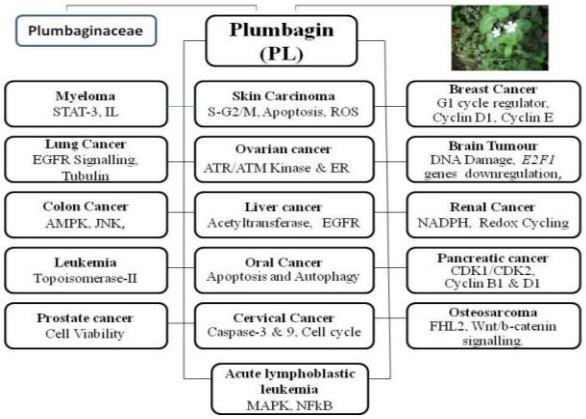
PL promotes cellular apoptosis and autophagy in TSCC cells involving p38 MAPK- and PI3K/Akt/mTOR-mediated pathways with contribution from the GSK3β and ROS-mediated pathways.[13]

15. Osteosarcoma: Osteosarcoma, a primary bone sarcoma is generally observed in children and adolescents and comprises ~5% of paediatric tumours and ~20% of bone tumours with predominance in males.[57] PL has shown to suppress the expression of FHL2 and exhibited significant anti-proliferative activity in osteosarcoma cells. It also attenuated Wnt/β-catenin signalling by down-regulating β-catenin and its target genes. PL also dose dependently reduced the expression of FHL2 in osteosarcoma U2OS, HOS, SaOS2 and MG63 cells, including c-Myc and WISP-1[58] By contrast, HOS cells expressed a lower amount of FHL2 and exhibited almost insignificant levels of FHL2 following PL exposure and negligible expression of c-Myc and WISP-1. PL significantly reduced the proliferation of osteosarcoma cells via the inhibition of Wnt/β-catenin signalling by downregulating the Wnt co-regulator FHL2, an oncoprotein in osteosarcoma cells. Therefore, with additional preclinical and clinical evaluations, PL may be effectively used in osteosarcoma therapeutics.[59]

16. Skin carcinoma: The anticancer effects of PL have also been demonstrated against human melanoma cells. In A375.S2 cells, PL was found to induce apoptosis and S-G_2/M cell cycle arrest leading to inhibition of cell growth. PL treatment was found to result in elevated level of p21 and reduced levels of cyclin B1, cyclin A, Cdc2, and Cdc25C. It also induced a change in Bax/ BCL-2 ratios and activation of caspase-9 resulting in apoptotic cell death. Using A-431 cells, the biological action of PL was reported through a mechanism involving redox recycling of transition metal ion copper. Studies involving scavenger of ROS and Cu (I)-specific chelating agent, viz. neocuproine, established that the anticancer action of PL involves generation of ROS and Cu(I), which serve as crucial mediators of PL-induced effects. PL was shown to inhibit proliferation and induce apoptosis in SKBR3 and BRCA cells, thus demonstrating an anticancer effect of PL against multiple cancers. The anticancer ability of PL against melanoma cells has also been demonstrated by other investigators.[11,60]

Conclusion

PL is a secondary plant metabolite and a pleotropic drug having wide variety of pharmacological actions. PL has capacity to inhibit number of cyclic constituents and pathways which are involved in the growth, development and multiplication of cell and whose activity is increased in cancerous cell. These include cytokines like interleukins, NF-kB, TNF-alpha, ROS, PI3K & p38 MAPK, DNA degradation, JNK, PKc, and STAT-3, mTOR, AKT. Hence, PL can be a potential therapeutic agent which can treat different types of cancer in all possible ways. Further studies are needed for development of its more active analogues and its clinical implication.



Anticancer Mechanism of Plumbagin in different type of cancer

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