Food and Chemical Toxicology 125 (2019) 566–582

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Emerging role of plumbagin: Cytotoxic potential and pharmaceutical relevance towards cancer therapy

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ARTICLE INFO

Keywords:
Plumbagin
Cancer
Cytotoxicity
Pharmaceuticals applications
Drug resistance

ABSTRACT

Plumbagin is a naphthoquinone derived yellow crystalline phytochemical. Plumbagin has a wide range of biological effects including cytotoxicity against cancer cells both *in vitro* and *in vivo*. Due to the pleiotropic nature of plumbagin, it shows the anticancer effect by targeting several molecular mechanisms including apoptosis and autophagic pathways, cell cycle arrest, anti-angiogenic pathways, anti-invasion and anti-metastasis pathways. Among many signaling pathways the key regulatory genes regulated by plumbagin are NF-kβ, STAT3, and AKT, etc. Plumbagin is also a potent inducer of ROS, suppressor of cellular glutathione, and causes DNA strand break by oxidative DNA base damages. *In vivo* studies suggested that plumbagin significantly reduces the tumor weight and volume in dose-dependent manner without any side effects in tested model organisms. Another exciting aspect of plumbagin is the ability to re-sensitize the chemo and radioresistant cancer cells when used in combination or alone. Nano encapsulation of plumbagin overcomes the poor water solubility and bioavailability obstacles, enhancing the pharmaceutical relevance with better therapeutic efficacy. Moreover, plumbagin can be introduced as a future phytotherapeutic anticancer drug after fully satisfied preclinical and clinical trials.

1. Plumbagin

Phytochemicals obtained from plants play a very prominent role both in the traditional as well as modern medicinal systems (Baker et al., 1995). Varieties of secondary metabolites isolated from plants have been documented in system as well as in modern drug with medicinal properties including anticancer (Kuete and Efferth, 2011). Despite, only a few from those phyto products could be introduced as an anticancer drug alone or in combination. *Plumbago zeylanica* L. (family Plumbaginaceae) commonly known as 'Chitrak' is a semi-climbing sub herb grown in most of rural India (Chopra and Nayar, 1956). There are ten genera in Plumbaginaceae family mainly grown in semiarid regions of the Mediterranean and central Asia. Mostly, three species of Plumbago plant are found in India including *Plumbago capensis*, *Plumbago rosea*, and *Plumbago zeylanica*. All the three species can be distinguished by naked eyes by their flower color, which is blue (*P. capensis*), red (*P. rosea*), and, white (*P. zeylanica*) respectively (Fig. 1). This plant is well-known for its medicinal properties in Indian traditional medicinal system (Chaplot et al., 2006; Modi, 1961). Areal parts of the plant are used to treat many diseases including rheumatic pain, scabies, sprains, and skin (Raman et al., 1999). The root of the plant and its component showed anticancer, anti-atherogenic, antioxidant, cardiotonic, central nervous system stimulating properties, hepatoprotective, and neuroprotective activity (Bopaiah and Pradhan, 2001; Sundari et al., 2017; Tilak et al., 2004).

Plumbagin (5-hydroxy-2-methyl-1, 4-naphthoquinone), a yellow crystalline phytochemical isolated from the roots of plant *Plumbago zeylanica* L. (Sandur et al., 2006) exhibited anti-proliferative, anti-angiogenic, anti-invasive, and apoptotic property in many human cancers *in-vivo* and *in-vitro* (Kuete et al., 2016; Nguyen et al., 2004). Available data suggested that plumbagin induced toxicity against cancer at very less concentration compared with chemical chemotherapeutics and had an excellent impact on human health through the neuroprotective property (Yuan et al., 2017). *In vitro* treatment of plumbagin showed toxicity against cancer cells as well as normal human immortal keratinocyte cell HaCaT (Inbaraj and Chignell, 2004). *In vivo* administration in xenograft mouse models is not showing any side effects in treated mice with a significant reduction in tumor growth (Kang et al., 2017). A detailed study revealed the mechanism of plumbagin induced cytotoxicity in cancer cells through modulating the genes involved in angiogenesis (including STAT3, TGF-b, interleukin-1a, NF-kB, and VEGF), proliferation, cell cycle arrest (p53, p21, cyclin A, Cdk2, and cyclin D1), generation of reactive oxygen species (ROS), etc. and their downstream regulated pathways (Hsu et al., 2006; Kuo et al., 2006; Park et al., 2013; Wang et al., 2008; Xu and Lu, 2010; Zhang et al., 2016). Studies also suggested that plumbagin can be used in combination with existing

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https://doi.org/10.1016/j.fct.2019.01.018
Received 31 October 2018; Received in revised form 4 January 2019; Accepted 20 January 2019
Available online 24 January 2019
0278-6915/

Fig. 1. Plumbagin plant species mainly grown in India. (A) *Plumbagin zeylanica,* **(B)** *P. zeylanica* (white flower), **(C)** *P. zeylanica* (root), **(D)** *P. zeylanica* (seed), **(E)** *Plumbagin capensis* (blue flower), **(F)** and *Plumbagin rosea* (red flower). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

anti-cancer drugs that would be helpful in the treatment of radiotherapy and chemotherapy-resistant patients (Nair et al., 2008; Sandur et al., 2010). So, plumbagin can be used to overcome this obstacle by making cancer cells to sensitize against existing chemotherapeutic drug alone or in combination without causing any side effects. Several basic research on this phytochemical as an anticancer agent are already available. Still, this compound has not been in the market for the treatment of cancer. Therefore, the objective of this review is to provide a through light on this potent phytotherapeutic compound and to attract the pharmaceutical industry to carry out the clinical trials. To compile all the available data on plumbagin, we have used different e-sources like pubmed, google scholar, science direct, research gate etc. To date; a review on plumbagin can increase our knowledge and understanding of this potent phytochemical. This review will also establish an excellent route so that it can be introduced in next-generation anticancer drugs and would be beneficial for human beings suffering from deadly cancer.

2. Plumbagin and its analogue

Plumbagin (5-hydroxy-2-methyl-1, 4-naphthoquinone), a plant-based yellow crystalline secondary metabolite belongs to Quinone family. Mostly, it is isolated from the root of *Plumbago* plant but also found in root, leaf and stem bark of some other plants belonging to family of Ancestrocladaceae, Dioncophyllaceae, Droseraceae, and Ebenceae (Padhye et al., 2012). Plumbagin is a vitamin K3 analog having chemical formula $C_{11}H_8O_3$, and molecular weight 188.18 g/mol. Cytotoxic nature of Plumbagin is due to its unique structure with Quinone ring that involves in radical formation via electron transport in the presence of Quinone ring. Chemical structure of plumbagin and its natural (Fig. 2A) as well as synthetic (Fig. 2B) analogs are given below-

3. Plumbagin in cancer

Cancer is the fastest growing, group of disease involving uncontrolled growth of cells leading to death at the end. Cancer of colorectal, liver, lung, prostate, and stomach commonly occurs in male while breast, cervical, colorectal, lung, stomach, and uterine, are very frequent in the female. Generally, three treatment options are frequently used for cancer, which are chemotherapy, radiotherapy, and surgery either alone or in combination. Chemotherapy is before most effective among the three cancer treatment options. Many chemotherapeutic drugs are available for treatment of cancer, but none of them can be claimed as permanent cure. And, major drawbacks of all these drugs are serious side effects, expensiveness, and development of resistance. Therefore, the discovery of some novel and potent anti-carcinogenic drug with minimal side effects are in demand of the hours. Compounds isolated from natural resources may have high therapeutic efficacy, minimal side effects and lesser toxicity for normal cells

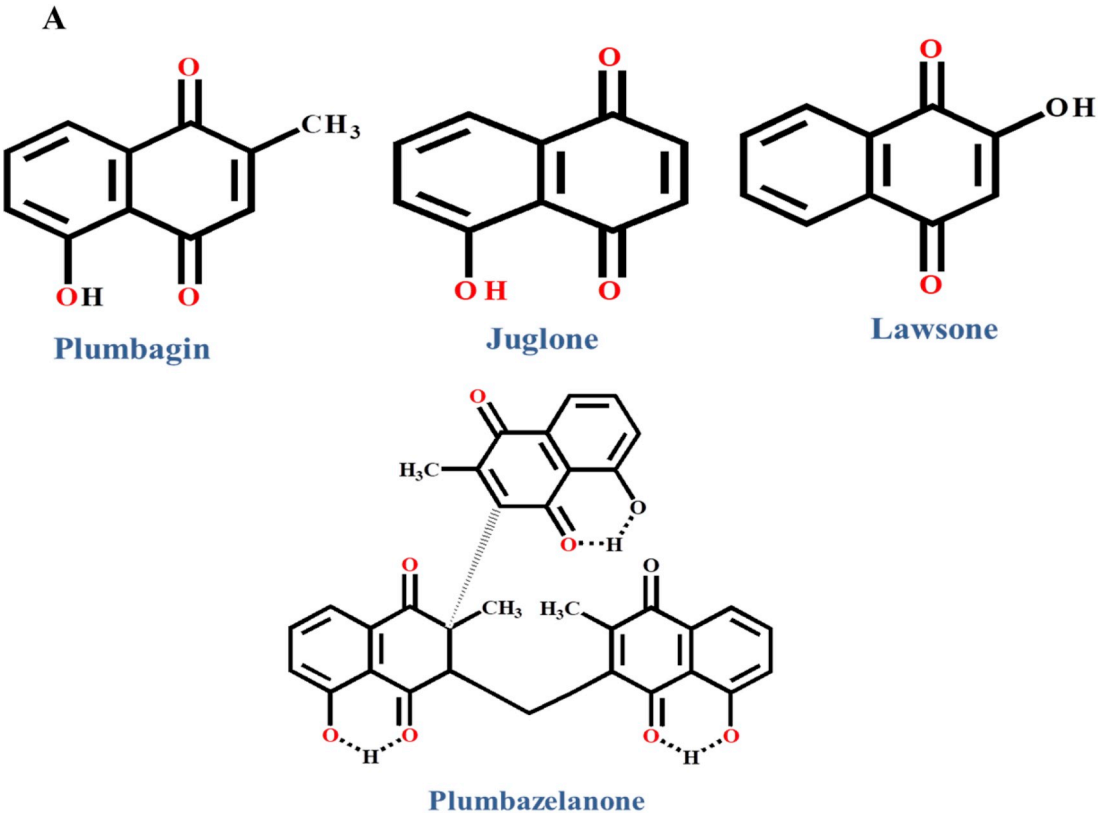

Fig. 2A. Chemical structures of Plumbagin and its natural derivatives.

compared to existing chemotherapeutics. So, this review focuses on a natural naphthoquinone derivative phytochemical named plumbagin, well known for its high therapeutic efficacy, pharmacological relevance, and minimal side effects as an anti-carcinogen (Tandon and Kumar 2013). A summarized form of *in vitro* cytotoxic effect and regulatory mechanisms of plumbagin on cancer is given in Table 1. Detailed regulatory impacts of plumbagin in different types of cancer are discussed below-

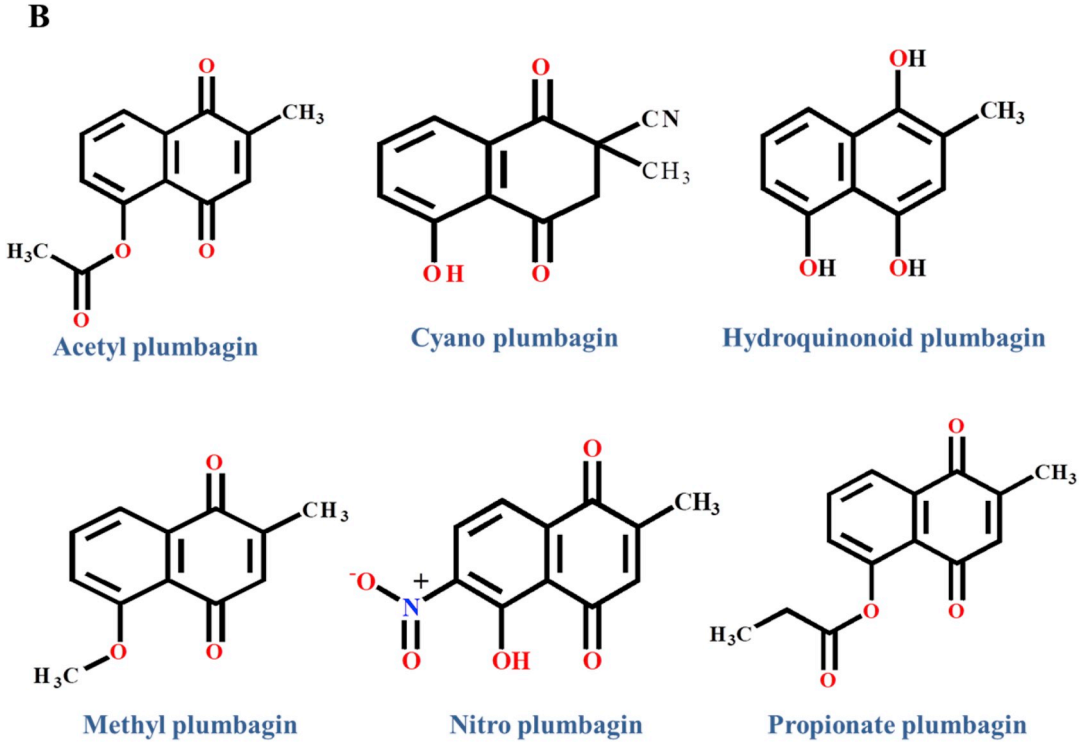

Fig. 2B. Chemical structures of synthetic derivatives of plumbagin.

Table 1
In-vitro cytotoxic effect and regulatory mechanisms of plumbagin on cancer.

SI. No.	Cancer	Cell type	Cytotoxic concentration (IC50) & time	Observation	Ref
01.	Lung cancer	A549 H292 H460	10.3 μM/12 h 7.3 μM/12 h 6.1 μM/12 h	Apoptosis, autophagy, cell cycle arrest. NF-kβ ↓, ROS ↑, caspase-9 ↑, caspase-3 ↑, Bcl-2 ↓, Bax ↑, Bak ↑, and CytC ↑.	Xu et al. (2013)
02.	Breast cancer	MM-468 MM231	2.03 μM/24 h 9.91 μM/24 h	Apoptosis, autophagy, cell cycle arrest, and inhibited invasion, migration, and metastasis. NF-kβ ↓, IL-6 ↓, CCL-2 ↓, caspase-3 ↑.	Messeha et al. (2018)
03.	Cervical cancer	ME-180	3.0 μM/48 h	Apoptosis. ROS ↑, caspase-3 ↑, Bcl-2 ↓, Bax ↑, surviving ↓, caspase-9 ↑, CytC ↑, AIF ↑.	Srinivas et al. (2004b)
04.	Colorectal cancer	HT29 HCT15	22.5 μM/24 h 62.5 μM/24 h	Apoptosis, cell cycle arrest. TNFα ↓, caspase-3 ↑, CytC ↑, Akt ↓, EGFR ↓, and GSK-3β ↓.	Subramaniya et al. (2011)
05.	Esophageal cancer	KYSE150 KYSE450	6.4 μM/24 h 8.0 μM/24 h	Anti-proliferative and apoptosis. STAT3-PLK1-AKT ↓	Cao et al. (2018)
06.	Gastric cancer	SGC-7901 MKN-28 AGS	19.12 μM/24 h 13.64 μM/24 h 10.12 μM/24 h	Apoptosis, inhibited invasion and migration. NF-kβ ↓, IAP1 ↓, XIAP ↓, Bcl-2 ↓, Bcl-xl ↓, and VEGF ↓.	Li et al. (2012)
07.	Gliomas	U87 A172 SHG44 U251	5.52 μM/24 h, 3.08 μM/48 h, and 2.83 μM/72 h 10.88 μM/24 h, 7.63 μM/48 h and 6.86 μM/72 h 6.75 μM/24 h 3.21 μM/48 h, 3.02 μM/72 h 8.71 μM/24 h, 7.54 μM/48 h, 6.52 μM/72 h	Apoptosis and cell cycle arrest. Cyclin D1 ↓, Cdc25B ↓, FOXM1 ↓, p21 ↑, p27 ↑, and caspase-3 ↑.	Niu et al. (2015)
08.	Hepato-cellular carcinoma	Hep G2	4 μM/24 h and 3.5 μM/48 h	Inhibit invasion and migration. MMP-2 ↓, u-PA ↓, u-PAR ↓, TIMP-2 ↑, and PAI-1 ↑.	Shih et al. (2009)
09.	Leukemia	HL-60 Jurkat K562 Raji U937	1.38 μM/24 h, 0.92 μM/48 h, 0.90 μM/72 h 2.20 μM/24 h, 0.98 μM/48 h, 0.86 μM/72 h 1.07 μM/24 h, 0.90 μM/48 h, 0.89 μM/72 h 5.06 μM/24 h, 3.49 μM/48 h, 2.66 μM/72 h 0.82 μM/24 h, 0.68 μM/48 h, 0.66 μM/72 h	Apoptosis and cell cycle arrest. ROS ↑, GSH ↓, Bcl-2 ↓, Mcl-1 ↓, DR4 ↑, DR5 ↑, Bax ↑, Bak ↑, Bcl-xL ↓	(Fu et al., 2016; Gaascht et al., 2014)
10.	Multiple Myeloma	OPMI	50 μM/48 h	Apoptosis and cell cycle arrest. STAT3 ↓, IL-6 ↓, PI3K/Akt-mTOR ↓, caspase-3 ↑.	Wu et al. (2016)
11.	Oral cancer	HSC-2 HSC-3 HSC-4 SAS OSC-19	9.02 μM/72 h 7.01 μM/72 h 4.34 μM/72 h 3.87 μM/72 h 14.6 μM/72 h	Apoptosis. ROS ↑, p53 ↓, Bax ↑, caspase-3 ↑, JNK ↓.	Ono et al. (2015)
12.	Osteosarco-ma	MG-63	15.9 μg/ml	Apoptosis and suppressed proliferation. c-myc ↓, (MDM2)/cyclin B1 ↓, cyclin D1 ↓, p21 ↑, p53 ↑.	Yan et al. (2015)
13.	Ovarian cancer	PEO-1 PEO-4	5 μM/24 h	Apoptosis and inhibited angiogenesis. VEGF ↓, Glut-1 ↓, p53 ↑.	Sinha et al. (2013)
14.	Pancreatic cancer	PANC-1 BxPC-3	9.9 μM/24 h and 7.0 μM/48 h 8.4 μM/24 h and 5.9 μM/48 h	Autophagy and cell cycle arrest. PI3K/Akt/mTOR ↓, p38 MAPK ↓, AMPK ↑, E-cadherin ↑, N-cadherin ↓, beclin1 ↑, ROS ↑, EGFR ↓, STAT3 ↓, MMP9 ↓, surviving ↓, caspase-9 ↑, caspase-3 ↑, Bax ↑.	Wang et al. (2015)
15.	Prostate cancer	PC-3 DU145	15.9 μM/24 h and 13.6 μM/48 h 6.9 μM/24 h and 4.8 μM/48 h	Apoptosis, autophagy, cell cycle arrest, inhibited invasion and metastasis. Sirt1 ↓, ROS ↑, PI3K/Akt/mTOR ↓, p38 MAPK ↓, cdc25A ↓, Bcl-xl ↓, caspase-9 ↑, caspase-3 ↑, caspase-8 ↑.	Qiu et al. (2015)
16.	Renal cancer	HEK293 LN229	2 μM	Inhibited the superoxide production. Nox-4 ↓	Ding et al. (2005)
17.	Melanoma	A-431	25 μM/24 and 48 h	Apoptosis and cell cycle arrest. cyclin B1 ↓, cyclin A ↓, cdc2 ↓, cdc25C ↓, Bax/Bcl ↑, caspase- 9 ↑, ROS ↑, caspase-3 ↓, PI3K/Akt/mTOR ↓.	Nazeem et al. (2009)

3.1. Plumbagin in lung cancer

Lung cancer is the leading cause of mortality and morbidity of human all over the world (Miller et al., 2016). Current treatment policies for lung cancer are not much more effective due to side effects, severe toxicity, and resistance development. Kang et al. demonstrated the impact of plumbagin in A549 lung cancer cells and BalB/c mice by targeting osteopontin regulated Rho-associated kinase signaling pathway (Kang et al., 2017). Osteopontin was found to inhibit the activity of cofilin (involved in actin polymerization and depolymerization of migratory cells) and promoted non-small cell lung cancer motility and invasiveness through focal adhesion kinase (FAK)/AKT/Rho-associated kinase (ROCK) pathway (Kang et al., 2015). Plumbagin treatment on osteopontin treated lung cancer cells (A549 & H1299) and in BalB/c nude male mice significantly reduced the motility, invasiveness, and activity of cofilin. Subsequently, it downregulated the expression of ROCK1, and LIMK1/2 while, attenuating the elevated phosphorylation level of cofilin, FAK, and AKT without any change in total forms. These observations indicated the cytotoxic, anti-invasion and anti-migration properties of plumbagin by targeting ROCK and cofilin in osteopontin treated lung cancer *in vitro* and *in vivo* without any side effects (Kang et al., 2017). Treatment of plumbagin in A549 cells induced apoptosis by G2/M phase arrest, overexpression of p21 and downregulation of cyclinB1, Cdc2, and Cdc25C. Moreover, partial blockage of p53 activity by transfection of dominant-negative p53 was unable to confirm whether p53 was involved in the G2/M arrest or not (Hsu et al., 2006). Further study suggested that plumbagin induced apoptosis through a change in Bax/Bcl-2 ratios, depolarization of mitochondrial membrane potential, caspase-9 activation and cytochrome C release. Also, cell growth inhibition property of plumbagin was due to activation of the c-Jun NH2-terminal kinase (JNK) because it enhanced the stability of p53 by reducing the interaction between p53 and MDM2. A higher number of TUNEL positive cells in A549 tumor xenograft mice compared to vehicle-treated mice confirmed the *in vivo* tumor growth inhibition and apoptotic property of plumbagin. Also, protein extracted from plumbagin treated A549 tumor xenografts showed an increased level of activated caspase-3, phospho-p53, phospho-JNK, cleaved PARP as compared to vehicle-treated mice (Hsu et al., 2006).

EGFR expression level has been examined as a cancer therapeutic approach in many types of cancer including lung cancer. The study suggested that plumbagin downregulated the EGFR/Neu expression and its downstream signaling such as Akt, NF-κB, Bcl-2 and survivin in lung cancer A549 and H460 cells (Gomathinayagam et al., 2008). It was also observed that plumbagin arrested the H460 cells in G2/M phase through up-regulated p53 and p21CIP1/WAF1 expression while downregulated cyclinB1 and Cdc25B proteins expression. Finally, plumbagin induced apoptosis in A549 and H460 cells through the activation of JNK/p8 and caspase-3 signaling pathways. Yan-Cong Li et al. suggested that plumbagin induced apoptosis in human non-small cell lung carcinoma (A549 and H23) via G2/M phase arrest and autophagic cell death by inhibiting the PI3K/Akt/mTOR pathway (Li et al., 2014). Plumbagin also induced the ROS production in both A549 and H23 cells leading to apoptosis. The study suggested that there is a cross-talk between autophagy and apoptosis in a coordinated manner. Inhibition or induction of autophagy promoted the plumbagin mediated apoptosis in non-small cell lung carcinoma.

Interestingly, plumbagin increased the intracellular ROS level and suppressed the nuclear translocation of NF-kB which inhibited the IkBk degradation in lung H460cancer cell (Xu et al., 2013). Exposure of plumbagin with N-acetyl glucosamine (ROS scavenger) on H460 drastically reverses the apoptotic effect, and NK-kB inactivation is supporting the ROS mediated apoptotic effect of plumbagin. The same study also suggested that the compound enhanced the activity of caspase-3 and caspase-9, and downregulated the expression of Bcl-2, while upregulated the expression of Bax, Bak, and cytochrome C. Matrix metalloproteinases (MMPs) and urokinase-type plasminogen activator (u-PA) actively participate in metastasis process and their overexpression leads to cancer metastasis (Huang et al., 2005). Shieh et al. investigated the anti-metastatic property of plumbagin on 12-O-tetradecanoyl phorbol-13-acetate (TPA) regulated MMPs and u-PA activation in A549 cell (Shieh et al., 2010). This study suggested the TPA inhibition ability leading to anti-adhesion and anti-invasion properties of plumbagin. Plumbagin had also shown the inhibitory property against extracellular signal-regulated kinase 1 and 2 (ERK1/2) which downregulated the TPA induced protein, RNA level of MMPs and u-PA. Further data suggested that compound blocked the phosphorylation activity of TPA, degradation of kappaBa (IkBa), c-Fos, c-Jun, and the nuclear levels of nuclear factor kappa B (NF-kB). A study is also available on plumbagin effect on NCI-H460 induced cancer in a xeno-transplanted zebrafish model. Oral administration of plumbagin in xeno-transplanted zebrafish model up-regulated the expression of p53 gene which was similar to study reported in mice model system (Vinothkumar et al., 2017). The interleukin (IL)-6/signal transducer and activator of transcription (STAT) 3 signaling pathway regulated many cellular activities such as angiogenesis, apoptosis, invasion, and proliferation. Compiling research evidence suggested the involvement of IL-6/STAT3 signaling pathway in anticancer research. Plumbagin treatment significantly reduced the IL-6/STAT3 signaling pathway genes including Bcl-2, VEGF, and CycD1 expression. This report suggested the anti-angiogenesis and anti-proliferative property of plumbagin through IL-6/STAT3 signaling pathway (Yu et al., 2018).

3.2. Plumbagin in breast cancer

Breast cancer is the most common type of cancer occurring in women of India as well as throughout the world (Malvia et al., 2017; Siegel et al., 2015). The heterogeneous nature of this cancer makes it complicated towards targeted therapy. Triple-negative breast cancer does not express estrogen, progesterone and the Her-2/neu (ErbB2) receptors. Plumbagin has pleiotropic nature due to which it can target both receptors positive and receptor-negative cancer cells. Ahmad A. et al. studied the effect of plumbagin on triple negative breast cancer cells and found that it suppressed the activation of Bcl-2 and DNA binding ability of NF-kB leading to apoptosis (Ahmad et al., 2008). A comparison study on treatment effect of plumbagin and taxol in triple negative breast cancer cells (MDA-MB-231 and MDA-MB-468) by Messeha S. S. et al. confirmed the superiority of plumbagin over taxon regarding the anticancer activity (Messeha et al., 2018). Plumbagin increased the activation of caspase-3 and downregulated the expression of chemokine C-C Motif Ligand 2 (CCL2) in a cell-specific manner (Messeha et al., 2018). Her2 is a well characterized oncogene belongs to epidermal growth factor receptor (EGFR) family occurring in the overexpressed condition in approximately ~30% of breast cancer. Her2 overexpression is directly related to poor prognosis, chemo-resistance, and metastasis. Treatment of plumbagin in Her2-overexpressed breast cancer cells (SKBR3 and BT474) induced mitochondrial-mediated apoptosis by G1 phase cell cycle arrest, caspase activation, and decreased the level of Bcl-2 protein (Kawiak et al., 2012). Plumbagin also exhibited anti-proliferative and autophagic cell death via G2/M phase cell arrest in human breast cancer cells (Kuo et al., 2006). This G2/M arrest was influenced by the increased expression of p21/WAF1 and Chk2 activation, reduced level of cyclin A, Cdc2, Cdc25C and cyclin B1. Autophagy-mediated cell death in breast cancer cells by plumbagin was corroborated by the use of autophagic cell death inhibitor named bafilomycin. Bafilomycin reduced the plumbagin induced autophagic cell death in breast cancer. Plumbagin has been found to regulate the survival signaling through the phosphatidylinositol 3-kinase/AKT pathway (Kuo et al., 2006). It inhibited the survival gene expression by inactivating the PI3-K/AKT signaling. Overexpression of AKT significantly reduced the plumbagin mediated autophagic cell death while blocking of AKT expression enhanced the autophagic cell death which confirmed the involvement of AKT in plumbagin mediated autophagy. *In vivo* study in nude mice with induced breast tumor of MDA-MB-231 cells supported the plumbagin mediated autophagic cell death via PI3K/AKT/mTOR pathways (Kuo et al., 2006). Also, molecular mechanism study in breast cancer demonstrated that plumbagin treatment

downregulated the expression of metastatic gene CXCR4 at the transcriptional level. Plumbagin mediated CXCR4 inhibition reduced the migration and invasion through inhibition of other metastatic gene CXCL12 mRNA expression and chromatin immunoprecipitation as well as NF-kB inactivation (Manu et al., 2011). Han Q. et al. demonstrated the synergistic effect of plumbagin and zoledronic acid on breast cancer cell MDA-MB-231SArfp. Plumbagin was found to be more effective in synergistic condition with zoledronic acid compared to alone. Both the drugs in combination altered the cytotoxic, anti-invasive and-anti-migratory effect in a better way by silencing Notch signaling pathways and Bcl-2 expression (Qiao et al., 2015). Interestingly, plumbagin targeted epithelial-mesenchymal transition (EMT) and inhibited the mesenchymal biomarker expressions leading to prevent breast cancer invasion. Another *in vitro* study also supported the anti-invasive and anti-migratory effect of plumbagin on MDA-MB-231SArfp cells by downregulating the expression of IL-1α, MMP-2, MMP-9, and TGF-β and inactivation of STAT3 (Yan et al., 2013). Moreover, *in vivo* study confirmed the metastasis inhibitory effect of plumbagin on breast cancer cells, osteolytic bone, and secretion of MMP-2 and MMP-9 (Yan et al., 2014).

Breast cancer resistance is a major problem for patients because approximately 50% of patients acquired resistance within 5 years of treatment. Plumbagin showed active cytotoxic activity against human endocrine-resistant breast cancer cell at micromolar concentrations. Also, plumbagin re-sensitized the tamoxifen resistance breast cancer cells for tamoxifen drug when used in combination with tamoxifen (Sakunrangsit et al., 2016). Overexpression of the glucose-regulated protein 78 (GRP78) is related to acquire resistance for anti-estrogen agents through inhibition of pro-apoptotic BH3-only protein, Bik. A similar study on plumbagin effect on overexpressed GRP78 breast cancer cells suggested that plumbagin re-sensitized estrogen-positive breast cancer cells to tamoxifen by GRP78 inhibition and upregulation of Bik (Kawiak et al., 2017). Interestingly, plumbagin inhibited targeted epithelial–transition (EMT) and downregulated the mesenchymal biomarker expression leading to prevent the invasion of breast cancer. These findings supported the use of plumbagin in combination therapy with other existing drugs which will provide a new height to current cancer treatment policies. Aldehyde Dehydrogenase 1 (ALDH1) is the best marker to identify the breast cancer stem cell population in BRCA1 defective condition. Plumbagin showed selective sensitivity for BRCA1-defective HCC1937 cells by reducing the ALDH1+ population. Lee et al. identified PI5K involved in ROS generation as a new molecular target for plumbagin in MCF-7 Human Breast Cancer Cells (Lee et al., 2012). Overall, plumbagin was found to be a suitable phytotherapeutic agent for the treatment of breast cancer with minimal or nil side effects (Somasundaram et al., 2016).

3.3. Plumbagin in cervical cancer

Cervical cancer is the fourth most leading cause of cancer-related death in women throughout the world (Bray et al., 2018). Plenty of research data supported the ROS mediated anti-carcinogenic activity of plumbagin. Plumbagin exhibits the cytotoxic effect in cervical cancer through mitochondrial-mediated ROS generation in human cervical cancer cell line (ME-180) as per time and dose-dependent manner (Srinivas et al., 2004b). Plumbagin induced mitochondrial-mediated ROS generation was characterized by the use of ROS scavenger named N-acetylcysteine and mitochondrial cytochrome C. Treatment with plumbagin influenced the morphological changes of ME-180 cells including exposure of phosphatidylserine towards the outer side from the inner side of the membrane, nuclear condensation, and DNA fragmentation. Activation of caspase-3, caspase- 9, release of apoptosis-inducing factor (AIF) and mitochondrial cytochrome *c* confirmed plumbagin-induced apoptosis via mitochondrial caspase-dependent and independent pathways (Srinivas et al., 2004b).

Furthermore, Jaiswal A. et al. study supported the cytotoxic effect of the plumbagin was mediated through ROS generation which leads to apoptosis, inhibition of metastasis, and epithelial to mesenchymal transition in human cervical cancer (SiHa and HeLa) cells (Jaiswal et al., 2018). Plumbagin treatment was able to decrease the viability through G2/M and S-G2/M phase cell cycle arrest leading to cell death in SiHa and Hela cancer cells respectively. Plumbagin treatment arrested the cells by alleviating the expression of cell cycle regulatory genes cyclin B1, A and E2 and CDK 1/2 along with elevating the Bax/Bcl2 ratio and cleavage of caspase-3/9, and PARP. Interestingly, it has also been reported that plumbagin induced anti-metastatic effect even at non-cytotoxic doses through regulation of components involved in the maintenance of extracellular matrix including MMP-2/9, E-cadherin, N-cadherin, β-catenin, and vimentin (Jaiswal et al., 2018). Radiotherapy is one of the most conventional treatment options for cancer treatment including cervical cancer. Despite, its use is limited due to the protection of normal tissue from the required dose and development of resistance. Surprisingly, treatment of radiotherapy in combination with plumbagin induced apoptosis at a very low dose of radiation in a cervical cancer cell (C33A) (Nair et al., 2008). Enhanced caspase-3 activity supported the caspase-dependent pathways mediated cytotoxic activity of this combination therapy (plumbagin and radiation) in C33A cells. Additionally, Bcl-2, Bax, and Survivin expression were also found to be modulated by the treatment of plumbagin in combination with radiation therapy.

3.4. Plumbagin in colorectal cancer

Incidence of colon cancer commonly known as colorectal cancer is increasing day by day which made it primary health concern to humans worldwide. Therefore, a search for more efficient and aggressive treatment strategy is highly needed. Treatment with plumbagin, a natural compound, in human colonic cancer cells (HT29 and HCT15) promoted the activation of caspases-3, cytosolic cytochrome C, NF-kB, and TNF-α while, suppressed the activity of cyclin D1, pEGFR, pAkt, pGsk-3b, and PCNA leading to apoptosis (Subramaniya et al., 2011). COX-2 expression was also downregulated by plumbagin treatment attributed that plumbagin induced apoptosis in colonic cancer cells through upregulated TNF-α and downregulated COX-2 expression (Subramaniya et al., 2011). Treatment of colorectal cancer cell HCT116 with plumbagin arrested the cells in the G1 phase at micromolar range which indicated the apoptosis of HCT116 cells.

A detailed study suggested that G1 phase arrest of HCT116 cells was mediated through mitochondrial-mediated ROS generation (Eldhose et al., 2014). Wnt signaling is well-known for its involvement in cancer progression, and migration. It was found to be downregulated on exposure of plumbagin in colorectal cancer cell SW620. Plumbagin treatment also induced the downregulation of several downstream coactivators of Wnt signaling including β-catenin, TCF7L2, p300, Bcl9l, c-Myc, vimentin, and cyclin D1. Interestingly, use of isogenic HCT116p53+/+ and HCT116p53−/− colorectal cancer cells proved that downregulation of Wnt signaling was p53-independent in human colorectal cancer cells (Raghu and Karunagaran, 2014).

3.5. Plumbagin in esophageal cancer

Esophageal squamous cell carcinoma (ESCC) is a deadliest malignant disease, so requires novel effective drugs for its complete cure. Natural compounds can be a possible option due to its active medicinal property with fewer or no side effects. Plumbagin is the most effective natural product which can reduce the proliferation rate and survival of ESCC cells (KYSE150 and KYSE450) *in vitro* as well as *in vivo.* It inhibits the growth of ESCC cells (through mitotic arrest, apoptosis, and reduction in colony forming ability of cancer cells), and KYSE150 xenograft tumors also (Cao et al., 2018). Molecular mechanism study on the effect of plumbagin exposure on esophageal cancer cells resulted in decreased expression of transcription factors such as STAT3, polo-like kinase 1 (PLK1) and phosphorylated protein kinase B (p-AKT). Taken

together, reduced expression of these transcription factors induced apoptosis in esophageal cancer. Plumbagin suppressed cell viability and proliferation rate of ESCC cells through G0/G1 phase arrest without showing any cytotoxicity on normal esophageal cells. G0/G1 phase arrest of ESCC cells was mediated by upregulation of p53 and p21 and myeloid leukemia cell differentiation protein (Mcl-1), and down-regulation of cyclin D1, and CDK-4 (Cao et al., 2018). Similar to the previous finding, plumbagin exposure on ESCC cells showed cytotoxicity through STAT3 inactivation that was not observed on over-expression of STAT3. Plumbagin administration in ESCC xenograft mouse model revealed the delayed ESCC tumor growth with a significant reduction in STAT3 phosphorylation (Cao et al., 2018). These finding highly supported the anti-carcinogenic role of plumbagin in esophageal cancer through inhibiting STAT3 activation *in vitro* as well as *in vivo*.

3.6. Plumbagin in gastric cancer

Gastric cancer, a well-known cancer of the stomach is globally the third leading cause of cancer-related death (Bray et al., 2018). Its treatment is difficult because early stage symptoms are rarely appeared and usually diagnosed in an advanced stage. Therefore, more potent drugs are highly needed which can be useful in an advanced stage of gastric cancer. The study suggested that treatment of plumbagin on gastric cancer cell (MKN- 28) suppressed the activation of the JAK2/STAT3 pathway which was mediated through induction of SH2-containing protein tyrosine phosphatase 1 (SHP1) expression (Joo et al., 2015). Importantly, plumbagin reduced the expression of the cell cycle regulatory gene (including cyclin D1, Bcl-xl, and surviving), angiogenic factor named vascular endothelial growth factor 1 (VEGF1), and matrix metalloproteinase-9 (MMP-9) in gastric cancer (Joo et al., 2015). These are well-known factors involved in the activation of STAT3 in gastric carcinogenesis. Manu K. A. et al. suggested that plumbagin down-regulated the expression of CXCR4 expression in gastric and breast cancer that was found to be regulated at the transcriptional level (Manu et al., 2011). Plumbagin downregulated the CXCR4 expression by inhibiting its mRNA expression, NF-kB activation and CXCL12 expression leading to reduced migration and invasion of both gastric and breast cancer cells. A detailed study on the molecular mechanisms of the anti-carcinogenic effect of plumbagin in human gastric cancer cells (SGC-7901, MKN-28, and AGS) revealed that plumbagin induces *in vitro* cytotoxicity in a concentration-dependent manner. Plumbagin showed apoptotic properties by downregulating NF-κB expression and its downstream gene products such as Bcl-2, Bcl-xl, IAP1, tumor factor (TF), VEGF, and XIAP. In addition to this, plumbagin also suppressed the TNF-α mediated phosphorylation of p65 and IKK and IκBα degradation (Li et al., 2012). Overall, these finding strongly supported the cytotoxic, anti-proliferative, anti-migratory, and anti-invasive property of plumbagin in gastric cancer.

3.7. Plumbagin in gliomas

Gliomas are associated with primary brain tumors with high metastatic property in the surrounding area of the brain. The mortality rate of glioma patients is 97% within five years of diagnosis while survival rate in malignant glioma named glioblastoma multiforme is approximately 15–18 months (Bleeker et al., 2012). Plumbagin is a very potent anti-metastatic and anti-proliferative agent. Khawa A. K. et al. suggested that plumbagin causes DNA damage, cell cycle arrest and finally apoptosis in Human glioblastoma multiforme cells A172, KNS60, U251MG (KO) and medulloblastoma cell ONS76. These effects of plumbagin treatment were due to the upregulated activity of E2F1, TNFRSF1A caspase-3/7 genes and downregulated the E2F1 gene expression as well as a reduction in MDM2, cyclin B1, survivin and BCL-2 protein expression (Khaw et al., 2015). Importantly, plumbagin treatment in brain cells inhibited the telomerase activity which implies the cytotoxic effect of plumbagin through telomere shortening in brain tumor. Similar to other findings plumbagin treatment on Human glioma cells (U87 and U251) significantly reduced the migration and invasion of these cells by inhibition of MMP-2/9 expression, nuclear translocation of transcription factor Sp1 and also reduction of p-PI3K and p-Akt level in cells (Chen et al., 2017). These results were reversed on a combination treatment of plumbagin, and PI3K/Akt agonist insulin-like growth factor-1 (IGF-1) confirming the anti-migration and anti-invasion properties of plumbagin.

In vitro study in human glioma cells (U87, A172, SHG44, and U251) determined that plumbagin targeted and down-regulated the expression of FOXM1 and its downstream pathway genes such as cyclin D1 and Cdc25B as well as upregulated the expression of p21 and p27 (Niu et al., 2015). This finding supported the anti-carcinogenic potential of plumbagin by downregulating FOXM1 expression in gliomas. Further, *in vivo* administration of plumbagin in glioma cell xenografts in nude mice reduced the tumor volume by 54.48% compared with control without any side effect signs in treated mice (Niu et al., 2015).

3.8. Plumbagin in hepatocellular carcinoma

Hepatocellular carcinoma commonly known as liver cancer or primary hepatic cancer is the fourth most leading cause of cancer-related death globally (Bray et al., 2018). Shih Y. W. et al. have shown the anti-adhesion, anti-migration and anti-invasion abilities of plumbagin in liver cancer HepG2 cells. Degradation of the extracellular matrix (ECM) plays a prominent role in cellular invasion and migration. Therefore, treatment of plumbagin resulted in the reduction of genes involved in ECM stabilization (MMP-2, u-PA, and u-PAR) at mRNA and protein level, while elevating the level of TIMP-2 and PAI-1 (Shih et al., 2009). These finding supported the anti-metastatic properties of plumbagin in liver cancer. Next, treatment of plumbagin on HepG2 cells significantly down regulated the nuclear expression and binding abilities of NF-kB and AP-1 as per dose-dependent manner. Also, molecular mechanism study of plumbagin's effect on angiogenesis-mediated tumor growth in hepatocellular carcinoma (HCC) demonstrated the anti-angiogenesis properties of the plumbagin. Angiogenesis was suppressed by the inhibition of angiogenesis pathway's genes (PI3K-Akt, VEGF/KDR, and Angiopoietins/Tie2) expression and angiogenic factors including VEGF, CTGF, ET-1, bFGF in both cancer cells and xenograft tumor tissues (Wei et al., 2017). Plumbagin treatment in the co-cultured human endothelial cell (EA.hy926) and human hepatoma cells (SMMC-7721 and Hep3B) significantly reduced the viability, migration, invasion, and tube formation property which was induced by the hepatoma cells. In a similar *in vivo* study, oral administration of plumbagin (4 mg/kg of body weight) suppressed the 3- methyl-4-dimethyl aminoazobenzene (3Me-DAB) induced tumor in Wistar male rats. The expression level of glycolytic enzymes such as hexokinase, phosphoglucoisomerase, and aldolase was also found to be elevated in hepatoma-bearing rats that were almost maintained up to normal level in plumbagin administered rats (Parimala and Sachdanandam, 1993).

In contrast, some gluconeogenic enzymes including glucose-6-phosphatase and fructose −1, 6-diphosphatase which were present at low level in hepatoma-bearing rats; increased after plumbagin administration. These finding supported the anti-carcinogenic properties of plumbagin against 3Me-DAB induced tumor in Wistar male rats. Research data suggested that lysine acetyltransferases (KATs), p300 (KAT3B), and its close homolog CREB-binding protein (KAT3A) are closely associated with many cellular processes, so their deregulation leads to many disorder of human. *In vivo* study has been shown that plumbagin inhibited the histone acetyltransferase activity specifically the p300-mediated acetylation of p53 in a non-competitive manner (Ravindra et al., 2009). This regulatory effect of plumbagin on histone acetyltransferase activity signifies its role against many diseases including cancer.

3.9. Plumbagin in leukemia

Leukemia is another aggressive cancer with a high mortality rate due to poor prognosis. Plumbagin is characterized as an anti-proliferative agent leading to caspase-dependent apoptosis in leukemia. Its treatment in the human T-ALL MOLT-4 cells mediated the activation of mitogen-activated protein kinase (MAPK) pathways and inhibition of NF-kB signaling leading to apoptosis. Also, lipopolysaccharide (LPS) mediated phosphorylation of p65 and NF-kB target genes was inhibited by plumbagin treatment in MOLT-4 cells (Bae et al., 2016; Sandur et al., 2006). There was no significant cytotoxicity shown against normal peripheral blood mononuclear cells (PBMCs). Zhao Y. L. et al. showed the effect of plumbagin against acute promyelocytic leukemia (APL) derived NB4 cells and supported the anti-proliferative nature of the compound through G2/M phase cell arrest leading to apoptosis in a dose-dependent manner (2–15 μM/L) (Zhao and Lu, 2006). Histopathological study of tumor and organs from plumbagin (2 mg/kg body weight) treated NB4 tumor xenograft NOD/SCID mice demonstrated that tumor volume was reduced by 64.9% compared to control mice. There were no side effects (weight loss, tissue damage, and behavior change) observed in plumbagin treatment whereas side effects were seen in doxorubicin-treated mice (1 mg/kg body weight) in NB4 tumor xenograft NOD/SCID mice (Xu and Lu, 2010).

Mitochondria-mediated ROS generation is the best way of plumbagin induced cell death in cancer. Interestingly, it has been shown that plumbagin induced anti-proliferative and cytotoxic effect through excess ROS generation in leukemia K562 cells (2 μM) at very low dose compared to other cancer. Treatment with ROS scavenger NAC or PEG-catalase inhibited the plumbagin induced apoptosis, as well as increased expression of DR4 and DR5 confirming the ROS, mediated apoptosis in K562 cells (Sun and McKallip, 2011). Experimental research in HL-60 cells determined that plumbagin treatment enhanced the cleavage rate of DNA while this rate was significantly decreased by reduced activity of topoisomerase II activity (Kawiak et al., 2007). Moreover, pretreatment with NAC significantly reduced the DNA damage rate supported the indirect relation between ROS and DNA damage. Another study also supported the involvement of topoisomerase II-mediated DNA damage property of plumbagin when used in combination with shikonin (Fujii et al., 1992). The finding suggested that the DNA cleavage pattern induced by the combination of both the naphthoquinones was different compared to already existing topoisomerase II-active drugs. This DNA cleavage effect was highly suppressed when treated at an elevated temperature (65 °C) supported the involvement of topoisomerase II in naphthoquinones mediated DNA cleavage. An *in vitro* comparative study between chemotherapy drug of chronic lymphocytic leukemia (CLL), fludarabine and plumbagin revealed that plumbagin decreased cell viability and proliferation rate in HG3 and MEC-1 cells at a lower dose compared with fludarabine alone (Fu et al., 2016). Plumbagin treatment on HG3 and MEC-1 cells arrested both the cells in G0/G1 to S phase and S-phase respectively. Molecular mechanism study suggested that plumbagin mediated CLL cell apoptosis was due to a reduction in Bcl-2/Bax ratio.

Drug resistance is the most common problem in cancer treatment. Bui Thi Kim Ly et al. demonstrated the effect of plumbagin on imatinib-resistant chronic myeloid leukemia cells (TCCY or TCCY/T315I). Treatment of plumbagin on parental TCCY and imatinib-resistant chronic myeloid leukemia cells (TCCY/T315I) induced sensitivity in TCCY/T315I cells for plumbagin in a dose-dependent manner (Ly et al., 2017). Plumbagin also showed the anti-proliferative effect leading to apoptosis on both the wild-type Ba/F3 and the BCR-ABL-transfected Ba/F3 cells. These findings supported the re-sensitizing role of plumbagin in drug resistance myeloid leukemia. Compiling evidence suggested that many cancer cells become resistant to apoptosis through TRAIL signaling including TRAIL-R1 (DR4) and TRAIL-R2 (DR5) (Ly et al., 2017). Plumbagin treatment significantly increased the expression of DR4 and DR5 leading to cell death by solubilizing TRAIL.

3.10. Plumbagin in multiple myeloma

Multiple myeloma is a cancer of plasma considered as a treatable but most of the time not curable due to advanced stage diagnosis. The survival rate with current chemotherapeutic policies is usually 4–5 years due to its effect on many body parts. Experimental results indicated that plumbagin is a very prominent cancer cell cytotoxic agent and apoptotic inducer against multiple myeloma cell (OPM1) through enhanced caspase-3 activity and inhibition of PI3K, pAkt and p mTOR expression at the protein level (Wu et al., 2016). Similar to other cancers, plumbagin was recognized as an anti-angiogenic agent by inhibiting IL-6 mediated STAT3 phosphorylation and of c-Src, Janus-activated kinase (JAK) 1/2 activation in multiple myeloma also (Sandur et al., 2010). Plumbagin can also be used as a combination therapeutic in multiple myeloma because it was found that exposure of plumbagin on multiple myeloma cells potentiated the effect of thalidomide and bortezomib (Sandur et al., 2006). As per available reports, plumbagin mediated inhibition of NF-kB activation was related to TNF and some other inflammatory stimuli (such as cigarette smoke condensate, H_2O_2, IL-1b, LPS, OA, and PMA). TNF mediated suppression of NF-kB activation was correlated with inhibition of the expression of IkBa kinase, IkBa phosphorylation, IkBa degradation, p65 phosphorylation, p65 nuclear translocation. Also, plumbagin inhibited the direct interaction between nuclear p65 and of recombinant p65 to the DNA that was absent in the p65 plasmid (containing cysteine mutated at 38 residue position to serine) transfected multiple myeloma cells. Further study suggested that plumbagin has NF-kB-regulated apoptotic (Bcl-2, Bcl-xl, Bfl-1/A1, cFLIP, IAP1, IAP2, and survivin), anti-proliferative (cyclin D1 and COX-2), and anti-angiogenic (MMP-9 and VEGF) property in multiple myeloma (Sandur et al., 2006).

3.11. Plumbagin in oral cancer

Oral cancer is one type of head and neck carcinoma and fifth leading cause of cancer-related death in the world (Subapriya et al., 2007). So, novel therapeutic agents are a primary medical goal to provide better treatment option and improve the quality of oral cancer patient's life. Treatment of plumbagin in oral squamous cell carcinoma (OSCC) cells (HSC-2, HSC-3, HSC-4, SAS, and OSC-19) induced excess ROS generation leading to apoptosis through a decrease in mitochondrial membrane potential (Ono et al., 2015). Also, JNK phosphorylation and caspase-3/7 activity were significantly increased in plumbagin treated OSCC cells. The human tongue is the most common site for squamous cell carcinoma. *In vitro* treatment of plumbagin on human tongue carcinoma cell (Tca8113) suggested the growth inhibition and anti-proliferation activity of the natural compound through morphological changes and G2/M phase arrest (Qiu et al., 2013). A detailed study on molecular level suggested that G2/M phase arrest was due to an increased level of Bax/Bcl-2 ratio. Deshpande J. et al. study on the effect of plumbagin treatment in Indian patients derived oral cancer cells AW13516 (Dwivedi, DWD), AW 8507 (Gaurav) as well as one other oral cancer cell KB demonstrated the growth inhibitory effect of compound as per increasing concentration (10^{-7} M to 10^{-4} M) (Jyoti Deshpande et al., 2015). The molecular target of plumbagin in tongue squamous cell carcinoma (TSCC) cells (SCC25) was done by stable isotope labeling by amino acids in cell culture (SILAC)-based quantitative proteomic approach. And, the proteomic data analysis revealed about plumbagin inhibited targets including activated death receptor-mediated apoptotic pathway, cell proliferation, manipulated nuclear factor erythroid 2-related factor 2 (Nrf2)-mediated oxidative stress response signaling pathway and remodeled epithelial adherens junctions pathway (Pan et al., 2015b). Treatment of plumbagin arrested the cells at G2/M phase, reduced epithelial to mesenchymal (EMT) transition and stemness in SCC25 cells. More interestingly, plumbagin mediated translocation inhibition of Nrf2 from cytosol to nucleus supporting the promising anti-carcinogenic potential of the compound in SCC25 cells (Pan

et al., 2015b). Another study in SCC25 cells revealed the molecular mechanism of plumbagin induced apoptosis and cell cycle arrest. Plumbagin treatment elevated the Bax/Bcl-2 ratio, cell division cycle protein2 homolog (Cdc2) and cyclin B1 expression, in contrast, alleviated the p21 Waf1/Cip1, p27 Kip1, and p53 gene expression in SCC25 cells (Pan et al., 2015a). Additionally, plumbagin was also found as an autophagy inducer in SCC25 cells by inhibiting phosphatidylinositol 3 kinase (PI3K)/protein kinase B (Akt)/mammalian target of rapamycin (mTOR), glycogen synthase kinase 3β (GSK3β), and p38 mitogen-activated protein kinase (p38 MAPK) pathways (Pan et al., 2015a).

3.12. Plumbagin in osteosarcoma

Osteosarcoma is a frequently diagnosed primary bone tumor mainly in adolescent's age. This tumor is well known for poor prognosis and insensitive to chemotherapy. Treatment of osteosarcoma cells with plumbagin reduced cell viability and induced mitochondrial-mediated apoptosis via ROS generation, enhanced $Ca2^{+}$ release and endoplasmic reticulum (ER) stress and activated caspase3/9 (Chao et al., 2017). Another similar study in osteosarcoma cells MG-63 and U2OS also confirmed the cytotoxic growth inhibition effect of plumbagin through mitochondrial-mediated ROS generation. Plumbagin enhanced the expression of p53 and p21 in osteosarcoma cells while decreased murine double minute 2 (MDM2)/cyclin B1 and cyclin D1 expression (Tian et al., 2012).

Furthermore, Yan Chao-Hua et al. study in osteosarcoma MG-63 cells determined the growth inhibition property of plumbagin through DNA damage and S-phase cell cycle arrest. This cytotoxic effect was mediated by down-regulation of cyclin A, CDK2, and c-myc protein expression levels while upregulation of phosphorylated p53 and histone (Yan et al., 2015). Xue Y. L. et al. demonstrated the detailed molecular mechanism of plumbagin's antiproliferative activity. FHL2 (four and a half LIM domains 2), a multifunctional adaptor protein plays a crucial role in cell proliferation and differentiation, gene expression and signal transduction. Targeting plumbagin effect on FHL2 expression in various human osteosarcoma cell lines (such as HOS, MG-63, SaOS2, and U2OS) revealed that compound induced anti-proliferative activity by inhibiting the expression of FHL2 (Xue et al., 2016). Studies suggested that FHL2 has relation with Wnt/β-catenin signaling and its target genes such as v-myc, c-myc and WNT1 inducible signaling pathway protein-1 (WISP-1). Plumbagin treatment attenuated the Wnt/β-catenin signaling through suppressing the expression of β-catenin as well as it downstream target c-Myc and WISP-1 in osteosarcoma cells (Xue et al., 2016).

3.13. Plumbagin in ovarian cancer

Ovarian cancer is a cancer of ovary in females and mainly related to inherited or genetically transferred from women with BRCA1 or BRCA2 gene mutations (chance up to 50%) (Ford et al., 1995). Studies suggested that inhibition of BRCA1 or BRCA2 genes at mRNA and protein level enhanced the proliferation rate of ovarian cancer cells. Also, BRCA1 has a regulatory effect on estrogen receptor (ER) directly or indirectly. A comparison study between emodin, plumbagin, and tamoxifen, in BRCA1-blocked ER-positive BG-1 ovarian cancer cells demonstrated that only plumbagin showed statistically significant cytotoxicity. All the three drugs caused DNA fragmentation, loss of mitochondrial membrane potential, morphological changes and nuclear condensation in BRCA1-blocked BG-1 cells but plumbagin was found to be more efficient compared with other drugs (Srinivas et al., 2004a). Another comparison study between cisplatin, doxorubicin, plumbagin, and tamoxifen treatment in BRCA1 blocked ovarian cancer cell (BG-1) also supported the better cytotoxic and anti-carcinogenic potential of plumbagin compared with other existing drugs. Plumbagin can bind in the active site of ER-α; also induced estradiol-dependent expression of p53 and BARD1 in an ovarian cancer cell (NEO) (Thasni et al., 2008). These finding suggested the chemotherapeutic potential of plumbagin in BRCA1-blocked ER-responsive ovarian cancer patients. Angiogenesis is well known for tumor development, metastasis and drug resistance in almost all types of cancer. Sinha S. et al. showed the effect of plumbagin in ovarian cancer cells including cisplatin sensitive, BRCA2 deficient (PEO-1) and cisplatin-resistant, BRCA2 proficient (PEO-4) in normoxia and hypoxia condition. They demonstrated that both types of ovarian cancer cells were sensitive for plumbagin with any relevance of BRCA2 status (Sinha et al., 2013).

Interestingly, plumbagin inhibited the expression of VEGF-A and Glut-1 in PEO-1 and PEO-4 cells. It also suppressed the VEGF mediated pro-angiogenic signaling and endothelial cell proliferation. Additionally, an *in-vivo* study on plumbagin administration in OVCAR-5 tumor-bearing mice was associated with a reduction in Ki67, vWF and CD31 expression (Sinha et al., 2013). The overall finding confirmed the cytotoxic, antiproliferative, antiangiogenic effect of plumbagin in ovarian cancer *in vitro* as well as *in vitro*.

3.14. Plumbagin in pancreatic cancer

Pancreatic cancer is the highly aggressive and resistant malignancies in all over the world. It has 7th ranked in all type of human cancers in both male and females (Bray et al., 2018). Quick resistance against conventional cancer treatment options is a significant cause of limited therapeutic effect against pancreatic cancer. Chen et al. explored the anti-carcinogenic effect of plumbagin in pancreatic cancer cells PANC-1 and Bxpc-3. The compound treatment caused growth inhibition, morphological changes, Bax upregulation, deregulation in mitochondrial membrane potential, cleavage of procaspase-9 and overexpression of cytosolic apoptosis inducing factors. Plumbagin treatment activated caspase-3 only but not caspase-8 and pretreatment of caspase inhibitor did not affect the apoptosis of pancreatic cancer cells. Next, another possible way of plumbagin induced apoptosis might be due to downregulation of phosphoinositide 3-kinase activity through a negative feedback mechanism (Chen et al., 2009). *In vivo* study on plumbagin administration (2 mg/kg in DMSO) effect in orthotopic pancreatic tumor model observed the growth inhibition of Panc-1 xenografts without any toxicity (Chen et al., 2009).

Moreover, a finding also suggested that plumbagin significantly reduced cell viability through apoptosis induction in pancreatic cancer cells (PANC1, BxPC3, and ASPC1). Intraperitoneal treatment of plumbagin (2 mg/kg body weight, 5 days a week) in severe combined immunodeficiency (SCID) mice with PANC-1 cells induced ectopic tumor showed the significant reduction in tumor weight and volume without any observed side effects (Hafeez et al., 2012a). *In vitro* and the histopathological study showed that plumbagin treatment inhibited the constitutive activation of epidermal growth factor receptor (EGFR), pStat3Tyr705 and pStat3Ser727, DNA binding of Stat3, and physical interaction of EGFR with Stat3 in PANC-1 and xenograft tumors respectively (Hafeez et al., 2012a). NF-kB lost its phosphorylation as well as binding ability with DNA after treatment with plumbagin in both pancreatic cells (PANC1 and ASPC1 and xenograft tumors. Similarly, compound treatment inhibited the downstream pathway genes of Stat3 and NF-kB including cyclin D1, MMP9 and survivin. Also, in human pancreatic cancer (PANC-1 and BxPC-3 cells) plumbagin induces cell cycle arrest, autophagic cell death, and suppression of epithelial to mesenchymal transition (EMT) phenotype. Cell cycle regulatory effect was seen through modulation of a gene involved in cell cycle regulation including CDK1/CDC2, cyclin B1, cyclin D1, p21 Waf1/Cip1, p27 Kip1, and p53 (Wang et al., 2015). Plumbagin induced the autophagy in both PANC-1 and BxPC-3 cells was demonstrated by the increased phosphatase and tensin homolog, beclin 1 expression, and the ratio of LC3-II over LC3-I. Besides their inhibition, p38 mitogen-activated protein kinase (p38 MAPK) and phosphatidylinositol 3-kinase (PI3K)/protein kinase B/mammalian target of rapamycin as well as 5′-AMP-dependent

kinase activation supported the pro-autophagic property of plumbagin in pancreatic cancer cells (PANC-1 and BxPC-3) (Wang et al., 2015). Also, plumbagin inhibited epithelial to mesenchymal transition (EMT) phenotype property by upregulating the expression of epithelial marker (E-cadherin) and downregulating the expression of mesenchymal marker (N-cadherin) in pancreatic cancer.

3.15. Plumbagin in prostate cancer

Data show that prostate cancer is the second leading type of cancer in males throughout the world (Bray et al., 2018). Detection of prostate cancer at an early stage is frequently curable by surgery or radiation therapy, but in the advanced metastatic stage, there is no possible treatment option available. In prostate cancer cells (DU-145 and PC-3), plumbagin induced excess intracellular ROS generation through endoplasmic reticulum stress which led to be a significant reduction in cell growth and finally apoptosis (Huang et al., 2018). Importantly, *in vivo* study in plumbagin treated PCa xenograft mouse model showed similar results without exhibiting toxicity. Treatment with plumbagin showed ER stress in mice bearing human PCa xenografts (Huang et al., 2018). According to a pre-clinical study, plumbagin has property to improve the efficacy of androgen deprivation therapy (ADT) *in vitro* as well as *in vivo* in prostate cancer mice model. Plumbagin significantly decreased the androgen receptor levels *in vitro* and 1 mg/kg in sesame oil dose combined with castration treatment showed 50% tumor reduction *in vivo*. This combined treatment of plumbagin enhanced the survival rate of mice with little toxicity (Abedinpour et al., 2017). Another *in vivo* study in orthotopic human prostate cancer (PCa) cells induced xenograft mouse model documented that plumbagin (2 mg/kg of body weight intraperitoneal five days in a week) for 8 weeks treatment inhibited the growth and metastasis of prostate cancer cells (Hafeez et al., 2013). Histopathological study on organ isolated from orthotopic xenograft mouse model showed the inhibition of metastasis in liver and lymph nodes but not in lungs while control mice had metastasis foci in all liver, lungs, and lymph nodes. This inhibition of growth and metastasis of PC-3M cells was due to inhibition of angiogenesis markers (CD31 and VEGF), metastatic marker (MMP9, MMP2, and uPA), proliferative markers (Ki-67 and PCNA), PKCε, pStat3Tyr705, pStat3Ser727, Stat3 downstream target genes (survivin and BclxL) and induction of iNOS expression (Hafeez et al., 2013). Aziz, M.H. et al. suggested that selective anti-invasive and apoptotic property of plumbagin in PCa cells was not seen in immortalized non-tumorigenic prostate epithelial (RWPE-1) cells. Also, compound administration (2 mg/kg body weight) in the ectopic implantation of hormone refractory DU145 PCa cells caused 3 weeks delay in tumor development as well as reduced the 90% tumor weight and volume. There was no tumor growth after 4 weeks of discontinuation of plumbagin treatment. The study suggested that protein kinase C epsilon (PKCε) plays a master role in the progression and invasion of hormone refractory PCa and plumbagin inhibit the PKCε expression by targeting other transcription factors including AP-1, NFkB, and Stat3 or associating with protein kinases (e.g., Raf-1, MEK1/2, ERK1/2, p38 MAPK) (Aziz et al., 2008; Hafeez et al., 2015). Intraperitoneal injection of plumbagin (2 mg/kg body weight) in transgenic adenocarcinoma of mouse prostate (TRAMP) mice decreased the tumor size and urogenital apparatus weight at 13 and 20 weeks of injection and also inhibited the *in vivo* PCa development (Hafeez et al., 2012b). Plumbagin was shown to reduce cell viability leading to apoptosis through ROS generation by modulating the expression of superoxide dismutase 2 (Mn-SOD) in human prostate cancer cells (PC-3, LNCaP, and C4-2) (Powolny and Singh, 2008). Another finding suggested that plumbagin treatment caused an imbalance of cellular redox status by depletion in primary non-protein thiol GSH. *In silico* study in human prostate cancer, PC-3 and DU145 cells predicted that plumbagin has interaction property with approximately 78 types of proteins involved in the cellular process including cell proliferation, signal transduction apoptosis, etc. Stable-isotope labeling by amino acids in cell culture (SILAC) in PC-3 and DU145 cells revealed that at least 1225, and 267 proteins were interacting with plumbagin while 341 and 107 signaling pathways were regulated by compound respectively (Qiu et al., 2015). Plumbagin restricted these pathways and had a regulatory effect on apoptosis, autophagy, cell cycle regulation, epithelial to mesenchymal transition (EMT), and reactive oxygen species generation (Qiu et al., 2015).

3.16. Plumbagin in renal cancer

Studies suggested the involvement of ROS in the initiation and/or progression of cancer despite, excess ROS might be used as an anticancer agent. NAD(P)H oxidase has been involved in excess superoxide (ROS can be one type) generation, and from different isoforms of NAD(P)H oxidase NOX-4 is highly expressed in vascular cells and tissues of kidney and brain. Cytotoxic effect of plumbagin treatment on the activity of Nox-4 in Human embryonic kidney 293 (HEK293) and brain tumor (LN229) cells was demonstrated in renal cancer (Ding et al., 2005). The activity of Nox-4 was significantly checked by plumbagin treatment as per time and dose-dependent manner in both HEK293 and LN229 cells. NAD(P)H oxidase inhibitor (diphenyleneiodonium) treatment in HEK293 confirmed the superoxide production activity of NAD(P)H oxidase. Next, a plumbagin mediated decrease in superoxide production in Nox-4 transfected COS-7 cells confirmed the plumbagin mediated inhibition of Nox-4 activity through direct interaction (Ding et al., 2005).

3.17. Plumbagin in melanoma

Melanoma is most frequently known as skin cancer generally cause of 80% death related to skin cancer (Bray et al., 2018). The frequency of skin cancer is increasing at a very fast rate. It develops resistance towards radiotherapy and conventional chemotherapeutics in the early stage of treatment. Therefore, novel therapeutics is needed to overcome the problem in skin cancer treatment either alone or in combination. Many studies are available which suggested that plumbagin can be used as an up-and-coming natural anti-cancer agent in combination with existing chemotherapeutics, radiation therapy or alone. Use of plumbagin in combination with radiation in chemo and radio-resistance melanoma cells B16F1 resulted in increased ROS level compared to chemo and radiation treatment alone. Plumbagin in combination with radiation also promoted the radiation mediated DNA damage and G2/M phase cell cycle arrest. As usual, this elevated ROS level and G2/M phase arrest was due to depletion in mitochondrial membrane potential and alleviated the level of Bax, cytochrome C, p53 expression, and Caspase-3 cleavage. Overall, plumbagin can be used as a radio-sensitizer for melanoma cancer cells (Rao et al., 2015). Combination of plumbagin with chemotherapeutic celecoxib has synergistic cytotoxic effect against melanoma cells through inhibition of COX-2 and STAT3 pathways. The administration of this drug combination in UACC 903 or 1205 Lu melanoma cells induced tumor in female Athymic-Foxn1 nude mice showed the reduction in xenograft melanoma tumors by 63% with almost negligible toxicity *in vivo*. So, these findings strongly supported the combination potential of plumbagin against cancer drug resistance (Gowda et al., 2017). Wang et al. study proved that cytotoxicity of plumbagin was due to S-G2/M phase arrest as well as elevated level of ROS generation through mitochondrial dysfunction in A375.S2 human melanoma cells (Wang et al., 2008). Cell cycle arrest was associated with enhanced p21 expression and decreased cyclin A, cyclin B1, Cdc2, and Cdc25C expression. Mitochondrial mediated apoptosis was confirmed by increased Bax/Bcl-2 ratio, leading to caspase-9 activation. Moreover, apoptosis signal-regulating kinase 1, JNK and extracellular signal-regulated kinase 1/2 (ERK1/2) were activated by plumbagin treatment. These effects are reversed when ROS scavenger vitamin C and catalase, as well as specific inhibitors of ERK and JNK, were used confirmed the ROS and JNK has a critical role in plumbagin induced

apoptosis (Wang et al., 2008).

4. Plumbagin as a signaling modulator in cancer

Numerous studies have been reported that plumbagin exhibited anticancer activities against a variety of cancers by regulating multiple cancer signaling pathways. Due to pleiotropic nature plumbagin can modulate many signaling pathways. Among many signaling pathways, the key regulatory genes modulated by plumbagin are NF-kB, STAT3, and Akt. Plumbagin mainly modulates the transcription factor NF-kB and its regulated signaling including adhesion molecules, cyclo-oxygenase-2 (COX-2), cyclinD1, MMPs, Bcl-2, Bax, TNF, etc. In gastric and lung cancer plumbagin reduced the cell viability by inhibiting the NF-Kβ signaling, inactivating NF-kB/p65 nuclear translocation and phosphorylation of IκBα and IKKα (Li et al., 2012). Plumbagin was further shown to induce apoptosis and cell cycle arrest in esophageal cancer by inactivating STAT3 signaling which downregulates polo-like kinase 1 (PLK1) and phosphorylated protein kinase B (p-AKT) (Cao et al., 2018). Interestingly, Hafeez et al. reported that plumbagin had potential anticancer activity against pancreatic cancer by inhibiting the association of STAT3 with EGFR by inhibiting STAT3 phosphorylation and also inhibited NF-κB activation (Hafeez et al, 2012a). It has been reported that plumbagin inhibits breast cancer cells invasion and migration by inhibiting STAT3 and downregulating inflammatory cytokines (IL-1α, TGF-β) and metastatic marker genes (MMP-2 and MMP-9) (Yan et al., 2013). Plumbagin treatment in lung cancer cells L9981 and NL9980 downregulated IL6 expression leading to IL6/STAT3 activation (Yu et al., 2018). A similar type of *in vitro* study in gastric cancer suggested that plumbagin suppressed the activation of the JAK2/STAT3 pathway through induction of SHP1 expression. Anti-proliferating effect of plumbagin has wide range among signaling pathways (Joo et al., 2015). Mainly plumbagin induced G2/M phase arrest in almost all types of cancer. *In vivo* study in nude mice with induced breast tumor of MDA-MB-231 cells supported the plumbagin mediated autophagic cell death via G2/M phase cell cycle arrest and inhibition of PI3K/AKT/mTOR pathways (Kuo et al., 2006). Plumbagin showed anti-proliferative property in multiple myeloma by inhibiting PI3K/Akt/mTOR also (Wu et al., 2016). Likewise, plumbagin exhibited a significant anti-proliferative and apoptotic activity by ROS generation, G2/M arrest, inhibition of PI3K/Akt/mTOR, p38 MAPK and GSK3-β signaling pathways in human tongue squamous cell carcinoma cells (Pan et al., 2015a). Besides, the anti-proliferative property it also worked as an EMT inhibitor in human pancreatic cancer by inhibiting PI3K/Akt/mTOR signaling pathway (Wang et al., 2015). The study also reported that it affects Wnt/β-catenin signaling. In osteosarcoma, plumbagin treatment was found to be the inhibitor of four and a half LIM domains 2 (FHL2) which in turn inhibited Wnt/β-catenin signaling via suppression of β-catenin translocation to the nucleus (Xue et al., 2016). Raghu et al. have been reported that plumbagin exhibited cell viability reduction in human colorectal cancer via downregulation of several downstream coactivators of Wnt signaling including β-catenin, TCF7L2, p300, Bcl9l, c-Myc, vimentin, and cyclinD1 (Raghu and Karunagaran, 2014). Summarized form Plumbagin regulated cellular signaling pathways involved in cancer is given in Fig. 3.

5. Plumbagin and cancer drug resistance

There are generally three treatment options for cancer including chemotherapy, radiotherapy, and surgery. Chemotherapy is the most effective treatment approach regarding cancer despite, development of resistance makes it a failure and less prevalent. Apoptosis and autophagy are two main regulatory events induced by anticancer agents leading to cancer cell death. In most of the cases, cancer cell acquired resistance against conventional treatment options so, fails to apoptosis or autophagy. During this situation combination therapy would be another possible approach for reversing the developed drug resistance to the anticancer agent. The exciting aspect of plumbagin is its ability to re-sensitize the chemo and radioresistant cancer cells. However, the exact mechanism through which plumbagin re-sensitize the drug-resistant cancer cells are not entirely understood. Bui Thi Kim Ly et al. study suggested that plumbagin effectively inhibited the growth and induced apoptosis in normal and imatinib-resistance chronic myeloid leukemia cells (TCCY and TCCY/T315I).

Further study revealed that plumbagin could inhibit the activation of STAT3 and JAK1 phosphorylation, suppress the expression of cyclin D1, Bcl-xl, and VEGF. Also, sensitized the STAT3 overexpressing human multiple myeloma cells to bortezomib and thalidomide anticancer drugs (Sandur et al., 2010). The glucose-regulated protein (GRP) 78 overexpression confirmed the chemoresistance against anti-estrogen agents in breast cancer through downregulation of pro-apoptotic BH3-only protein, Bik. Plumbagin treatment in tamoxifen resistant MCF-7 and T47D estrogen positive breast cancer cells enhanced the sensitivity of these resistant cells towards tamoxifen by inhibiting the GRP78 expression and inducing the Bik expression (Kawiak et al., 2017).

Interestingly, plumbagin in combination with tamoxifen inhibited the growth of endocrine-resistant cells and suppressed the mesenchymal biomarker expressions at a very low concentration (Sakunrangsit et al., 2016). Additionally, plumbagin reverts the sensitivity for radiation in radio-resistant cervical cancer cell C33A leading to apoptosis when used in combination with radiation (Nair et al., 2008). The caspase-3 was also upregulated in C33A with modulation in the expression of apoptosis regulatory molecules Bcl-2, Bax, and survivin. A summarized form of the anticancer effect of plumbagin in combination with existing chemo and radiotherapeutics is given in Table 2. Overall, plumbagin would be introduced as a pharmacological agent for reverting the drug and radioresistance in cancer treatment either alone or in combination after clinical trials.

6. Toxicity of plumbagin

Natural products as medicine have so much interest because of its high therapeutic potential and its minimal side effects. Plant-produced secondary metabolite named naphthoquinones such as plumbagin has several biological effects including cytotoxicity against cancer cells. Plumbagin also showed cytotoxicity against human normal immortal keratinocytes cell (HaCaT) (Inbaraj and Chignell, 2004). Therefore, concern regarding safety purpose has been raised during use of plumbagin as an anticancer drug in human. There is no health hazard report available related to occupational exposure of plumbagin during its production and processing. Available literature on epidemiological studies or case reports did not mention any case on the association between plumbagin and cancer risk except few minor side effects including diarrhea, increased number of white blood cell and neutrophil counts increases the level of serum phosphatase and acid phosphatase in blood, hepatic toxicity, and skin rashes (Singh and Udupa, 1997). But, *in vivo* studies in xenograft mice model almost neglected the safety concern as future anticancer drugs after the clinical trials. Plumbagin has been observed for antimalarial activity through acute, subacute and oral dose administration *in vivo* and *in vitro* (Sumsakul et al., 2014). For *in vivo* study rats of both the genders were selected by researcher and acute, subacute and oral dose administration effect of plumbagin were observed. Plumbagin single oral dose of 150 mg/kg body weight was safe, and no visible toxicity sign was observed within 14 days of treatment. While, for subacute toxicity test, oral dose of 25 mg/kg body weight plumbagin was given for continuous 28 days and still no toxicity was observed in rats of both the genders. Histopathological analysis of compound-treated rat's organs (liver, kidney, heart, spleen, and lung) taken from both the experiment did not show any morphological changes like shape size, weight. Doses higher than 250 mg/kg body weight revealed toxicity sign and symptoms including agitation, anxiety, exhibited chewing, and salivation.

Cytochrome 450 is a large family protein which is involved in the removal of xenobiotic compounds from the body as well as metabolism

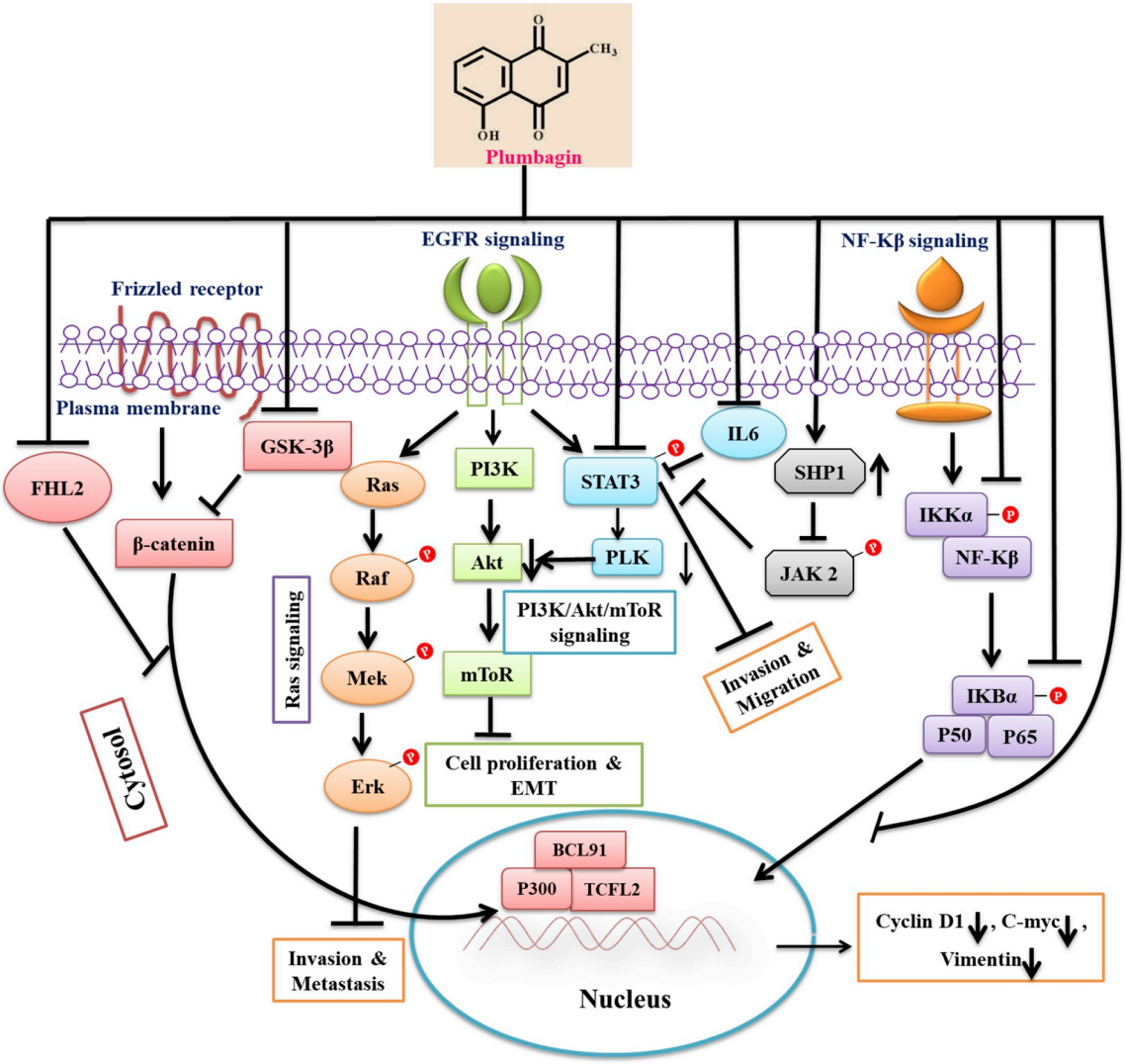

Fig. 3. Plumbagin regulated cellular signaling pathways involved in cancer.

of drugs. Plumbagin was not found any modulatory effect on mRNA expression of cytochrome 450 isoforms including CYP1A2 and CYP3A11 in rat livers. Overall, plumbagin showed satisfactory toxicity profile during *in vivo* oral administration in rats of both the genders (Sumsakul et al., 2016). Plumbagin was found to be a potent inducer of ROS, suppressor of cellular glutathione, and causes DNA strand break by oxidative DNA base damages. This compound can induce DNA damage at a nano-molar concentration (0.25 ng/ml) without affecting the viability of cells (Demma et al., 2009). Plumbagin administration in rat model at 1–16 mg/kg body weight concentration for 24 h showed that ascorbate and NADPH-dependent lipid peroxidation was inhibited while there was no effect on cumene hydro peroxide-dependent lipid peroxidation (Sankar et al., 1987). Liver glutathione (GSH) and microsomal glucose-6-phosphatase levels were also decreased by 16 mg/kg injection of the compound. Below non-DNA damaging concentration plumbagin significantly alleviated the catechol induced DNA damage. These findings strongly supported the antioxidant property of plumbagin. NF-kB is a well-known ubiquitous transcription factor that has regulatory effects on many cellular processes including cell proliferation, apoptosis induction of leukocytes, expression of immune regulatory genes, etc. Seshadri et al. reported that plumbagin induced apoptosis was caspase-3 dependent via mitochondria through ROS generation and GSH depletion in human peripheral blood lymphocytes (Seshadri et al., 2011). Plumbagin has been reported as a suppressor of NF-kB activation in many cancers which support the immunomodulatory effect of plumbagin leading to cytotoxicity against cancer cells (Checker et al., 2009). However, plumbagin analogs and derivatives also documented as cytotoxic and apoptotic inducer in cancer cells with less toxic effects on normal cells. Sagar et al., (2014) found that plumbagin derivative; acetyl plumbagin modulated the expression of many apoptotic genes leading to cell death in estrogen positive MCF-7 breast cancer cells with reduced toxicity on normal cells (Sagar et al., 2014).

Plumbagin induced cytotoxicity by two different mechanisms (1) redox cycling, (2) and reaction with glutathione (GSH) (Inbaraj and Chignell, 2004). Exposure of plumbagin with keratinocytes produced hydrogen peroxide (H_2O_2) leading to oxidation of GSH to GSSG while redox cycling generates semiquinone radicals. This depletion of GSH was found to increased H_2O_2 production, enhanced semiquinone radicals and high cytotoxicity (Inbaraj and Chignell, 2004). So, use of plumbagin without proper care may harm the skin. Plumbagin exposure on chick embryo fibroblast cultures showed powerful effects like inhibition of cell growth, proliferation, a less mitotic index with metaphase cell arrest. They suggested that at lower concentrations plumbagin works as a spindle poison while at high concentration it is nucleotoxic and cytotoxic (Santhakumari et al., 1980). In contrast, plumbagin treatment on breast cancer cells (MDAMB- 231 and MCF-7) and non-tumorigenic normal breast epithelial cells (MCF-10A) showed that

Table 2
Anticancer effect of plumbagin in combination with existing chemo and radiotherapeutics.

Sl. No.	Cancer	Cell Type	Drug combination & dose	Observation	Ref.
1.	Breast cancer	MDA-MB-231S Arfp	Plumbagin 7.5 μM with 50 μM zoledronic acid	Synergistically inhibited migration and enhanced apoptosis in breast cancer cells.	Qiao et al. (2015)
2.	Leukemia	Kasumi-1 cells	Plumbagin 2 μM with 100 ng/ml rsTRAIL.	Enhanced TRAIL-induced apoptosis by excess ROS, upregulation of DR5 expression, activation of caspase-8 and inhibition of cFLIP expression.	Kong et al. (2017)
3.	Gastric cancer	SGC-7901	Plumbagin 10 μM with 0.1 nM TNF-α and cisplatin	Enhanced apoptotic and cytotoxic effect of cisplatin against gastric cancer cells.	Li et al. (2012)
4.	Cervical cancer	C33A	Plumbagin 750 nM with 2 Gy of radiation	Induced higher apoptosis at a lower dose of radiation compared to a higher dose of radiation. Plumbagin may be potential radio-sensitizer acting through the induction of apoptosis.	Nair et al. (2008)
5.	Melanoma	UACC 903	Plumbagin 5 μM with 30–50 μM celecoxib.	Synergistically inhibited melanoma tumor growth by simultaneously targeting COX-2 and STAT3 pathways resulted in downregulation of cyclins.	Gowda et al. (2017)
6.	Prostate cancer	PTEN-P2	Plumbagin 0.5 or 4 μM with 10 − 8 M dihydrotestosterone (DHT)	Upregulated mRNAs caused by DHT were down-regulated by plumbagin and specifically downregulate cell cycle control genes and also regulate androgen receptor target genes.	Rondeau et al. (2018)

plumbagin induced apoptosis in breast cancer cells without affecting normal breast epithelial cells (Ahmad et al., 2008). Another study on plumbagin effect in prostate cancer cells demonstrated that plumbagin could kill the prostate cancer cells (DU145, CWR22rv1, and LNCaP) without affecting the immortalized prostate epithelial cell (RWPE-1) (Aziz et al., 2008).

Further, *in vivo* study demonstrated that the toxic side effects of plumbagin are dose and administration dependent. Toxic doses (LD50) of plumbagin which can induce side effect symptoms in mice through oral administration and intraperitoneal injection were 8–65 mg/kg body weight and 16 mg/kg body weight respectively (Sandur et al., 2006). While many *in vivo* studies in mice revealed that 2 mg/kg body weight intraperitoneal and 200 ppm in the diet of plumbagin is sufficient in the reduction of tumor weight and volume without showing any toxic side effects (Hafeez et al., 2013). Also, plumbagin had given negative Ames test result in *Escherichia coli* confirmed no mutagenic activity (Farr et al., 1985). Overall, finding suggested that plumbagin can be introduced as a plant-derived anticancer drug in combination or alone. A summarized form of preclinical studies on the anticancer effect of plumbagin in *in vivo* models is given in Table 3.

7. The pharmaceutical relevance of plumbagin

Natural products are diverse, unique and inspirational source of bioactive compounds which served as novel drugs. Despite, poor water solubility and bioavailability are significant drawbacks of plant-derived natural compounds. Plumbagin has high anti-carcinogenic potential while suffering from the same limitations including poor water solubility and bioavailability. Pharmacokinetics analysis of plumbagin single oral dose of in Wistar rats (100 mg/kg body weight) demonstrated that t1/2 (median elimination half-life), and MRT (mean residence time) of plumbagin was 9.6 and 5.0 h, respectively (Sumsakul et al., 2016). Plumbagin was observed up to 96 h of oral administration in the urine sample of Wister rats. Niosomes are mono-alkyl, nonionic surfactant derived vesicular structure enhanced the uptake capacity of intravenously administrated drugs by the liver. Raja Naresh R. A. et al. developed niosomal plumbagin, and his experimental analysis revealed that niosomal plumbagin had more antitumor activity with fewer side effects compared to free plumbagin (Naresh et al., 1996). Water solubility and drug availability for liver were also significantly enhanced.

Further, to increase the efficiency with reduced toxicity Kini D. P. et al. synthesized albumin microspheres of plumbagin and demonstrated the *in vivo* effect by intraperitoneally administration. Interestingly, antitumor and antifertility effects of plumbagin (5 mg/kg dose) were improved with a better survival rate of animal compared to niosomes control (Kini et al., 1997). Plumbagin nano-formulation was also found to be more cytocompatibility with normal cells as well as blood biocompatibility with minimal toxicity to prostate cells compared to crude compound extract. Plumbagin nano-encapsulation also showed cytotoxicity, anti-migratory, and DNA fragmentation property in prostate cancer cells. These finding indicated that plumbagin nanoparticles could enhance the anti-carcinogenic activity of plumbagin with better cytocompatibility and bioavailability (A Nair et al., 2015). Currently, colloidal silver nanoparticles (AgNPs) are used as a promising drug vehicle due to its chemical stability and low toxicity at a lower dose. Silver nano-encapsulation of plumbagin was shown to enhance its internalization in HeLa cells with inhibited proliferation rate at the IC50 value of 18 ± 0.6 μM (Appadurai and Rathinasamy, 2015). Also, clonogenic survival of cells was decreased, and cells were arrested in mitosis phase in a concentration-dependent manner. Duraipandy N. et al. caging the plumbagin in silver nanoparticles and their finding suggested that caging of plumbagin in silver nanoparticle improved the selectivity and sensitivity of the compound towards cancer cells. Silver nano caging also reduced the required dose for apoptosis up to 50% in cancer cells. Therefore, silver nanoparticles could overcome the limitation of poor solubility of and availability related problems of

Table 3
In vivo cytotoxic effect of plumbagin in different tumor models.

SI. No.	Animal Model	Cell Type	*In vivo* cytotoxic dose & route	Observation	Ref.
1.	Lung cancer BalB/c nude male mice	A549	5 mg/kg/day of body weight; intraperitoneally via tail.	Suppressed lung metastasis. No side effect in mice.	Kang et al. (2017)
2.	Breast cancer female nude mice (BALB/cA-nu)	MDA-MB-231	2 mg/kg of body weight; Intraperitoneally in flanks.	Tumor growth reduction, approx. 70% reduction in tumor size and an increase of autophagocytic vacuoles in the tumors. No side effect in mice.	Kuo et al. (2006)
3.	Colorectal cancer nude mice	HCT116	6 mg/kg/day of body weight; intralesional injection	Inhibited tumor growth and tumor angiogenesis by inhibition of VEGF-induced Ras/Rac/Cofilin and Ras/MAPK signaling pathways. No side effect in mice.	Lai et al. (2012)
4.	Esophageal cancer female xenograft nude mice (BALB/c-nu)	KYSE150	2 mg/kg/day of body weight; 5 days/week; intraperitoneally in flanks.	Inhibited tumor proliferation and increases apoptosis. No side effect in mice.	Cao et al. (2018)
5.	Gliomas female athymic nude mice	U87	2 mg/kg/day of body weight; 5 days/week; intraperitoneally	Reduced tumor volume by 54.48%; Inhibited cell- proliferation induced apoptosis by inactivation of FOXM1. No side effect in mice.	Niu et al. (2015)
6.	Leukemia male NOD/SCID mice	NB4	2 mg/kg/day of body weight; intraperitoneally.	64.49% tumor volume reduced and induced apoptosis in xenograft tumor model. No side effect in mice.	Xu and Lu (2010)
7.	Ovarian cancer female SCID mice	OVCAR5	1 mg/kg/day of body weight; intraperitoneally.	Inhibited tumor growth and angiogenesis confirmed by reduced tumor weight and volume and decrease in blood vessel formation in tumor respectively. No side effect in mice.	Sinha et al. (2013)
8.	Pancreatic cancer male SCID mice	PANC1	2 mg/kg/day of body weight; 5 days/week; intraperitoneally.	Inhibited tumor growth and phosphorylation and DNA-binding activity of NF-kB. No ide effect in mice.	Hafeez et al. (2012a)
9.	**a.** Prostate cancer male athymic nude mice	PC-3M-luciferase cells	2 mg/kg body weight; once a week and 5 days/week; intraperitoneally.	Inhibition of metastasis into the liver, but not observed in the lungs and lymph nodes. No side effect in mice.	Hafeez et al. (2013)
	b. Prostate cancer athymic BALB/cA nu/nu female mice	DU145	2 mg/kg body weight; once every other day; intraperitoneally.	Apoptosis through ROS-mediated ER stress pathway. No side effect in mice.	Huang et al. (2018)
	c. Prostate cancer Homozygous breeding pairs of TRAMP/FVB mice	Transgenic adeno-carcinoma of prostate	2 mg/kg body weight; 5 days/week; intraperitoneally.	The decrease in prostate tumor size and urogenital apparatus weights. No side effect in mice.	Hafeez et al. (2012b)
10.	Melanoma female athymic-Foxn^{1}nu nude mice	UACC 903 or 1205 Lu	0.5–1.0 mg/kg of body weight; on alternate days; intraperitoneally.	Inhibiting tumor growth and increased phosphorylation of PERK, eIF2a, and g-H2AX, with induction of CHOP and PARP cleavage. No side effect in mice.	Afaq et al. (2017)
11.	Lymphoma C57BL/6 mice	EL4	2 mg/kg of body weight; 5 days/week; intraperitoneally.	Apoptosis and decreased proliferation a by enhancing JNK phosphorylation. No side effect in mice.	Checker et al. (2015)

plumbagin up to the specific mark and enhanced its therapeutic values (Duraipandy et al., 2014). Despite, silver nanoparticles itself have restrictions regarding concentration-dependent toxicity and particle size. To overcome these limitations of silver nano-encapsulated plumbagin, Singh U. V. synthesized biodegradable polymeric poly (lactic-co-glycolic) acid (PLGA) injectable gel implant of plumbagin. PLGA gel implant of plumbagin enhanced the LD50 of plumbagin compared to only plumbagin which was 9.33 mg/kg and 8.60 mg/kg respectively. The toxicity of PLGA gel implant of plumbagin in mice after subcutaneous injection was also found to be much less compared to free plumbagin administration in BALB/c mice. Thus, the biodegradable gel implant is one of the best treatment options to overcome the poor solubility and availability problems (Singh et al., 1997).

Target drug delivery is one of the better treatment options for cancer due to its targeted effect without affecting other places which could minimize the toxicity of the drug. To make, targeted delivery of plumbagin, Minjie Pan M. Ma. et al. synthesized plumbagin-loaded poly D, L-lactic-co-glycolic acid-b-polyethylene glycol (PLGA-PEG) nanoparticles which targeted prostate-specific membrane antigen (PSMA) aptamer. Their finding suggested that targeted nanoparticles had more cytotoxicity and less circular half-life compared to the non-target nanoparticle. This also reduced the toxic adverse effect of free plumbagin with the enhanced therapeutic efficacy which is helpful to introduce the plumbagin in future anticancer drugs (Pan et al., 2017). Moreover, plumbagin can be introduced as a future anti-carcinogenic drug after fully satisfied preclinical and clinical trials.

8. Conclusion and future perspective

Currently, plant-derived compounds have more interest in the pharmaceutical industry for novel and potent drug development. Plumbagin, a plant-derived product has been demonstrated to exhibit various biological activities including anticancer property. It showed apoptosis, anti-proliferative, anti-invasive, anti-migration property and cell cycle arrest against various cancers by targeting multiple signaling pathways and excess ROS generation. Studies have been suggested the potential cytotoxic effect of plumbagin in *in vivo* models also without any visual side effects. Nowadays, a multi-target approach is highly needed for cancer treatment because of side effects and resistance development against current treatment policies. The emerging data on plumbagin as combination therapeutics with existing chemo and radiotherapeutics showed enhanced apoptosis and resensitized the resistance cells for existing drugs. Many efforts are going on to use plumbagin as a combination anticancer therapeutics. Poor water solubility and less bioavailability is the major problem for plant-derived drugs including plumbagin. Its nano formulation improved that without compromising its anticancer property. Despite, for human studies very strong and controlled clinical trials are still needed in a model system. Through this review, we have tried to pay attention to plumbagin *in vitro*, *in vivo* anticancer mechanisms, toxicology, pharmaceutical application, and pre-clinical trials, which need further studies to introduce plumbagin in future anticancer drugs.

Author contributions

Manuscript was prepared by SKT, graphical abstract; Table 3 and Fig. 3 were prepared by MP under the consistent guidance of BKB.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

The authors thank (a) MHRD and National Institute of Technology, Rourkela, Odisha for providing laboratory and fellowship to Surya Kant Tripathi; (b) and Department of Science and Technology, Science and Engineering Research Board (DST, SERB) (Grant Number: ECR/2016/000792) for providing fellowship to Munmun Panda.

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.fct.2019.01.018.

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