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Andrographolide and analogues in cancer prevention

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1. ABSTRACT

Andrographis paniculata is a medicinal plant traditionally used for treatment of cough and cold, fever, laryngitis, and several infectious diseases. Extracts of *A. paniculata* have shown versatile potency against various diseases including cancer. The active biomolecules of *A. paniculata* mainly are lactone and diterpene. Andrographolide and analogues have been widely used for prevention of different diseases. Andrographolides have shown potent antiinflammatory and anticancer activities. It showed potentials as chemopreventive agents by suppressing growth of cancer cells by inhibiting NF-κB, PI3K/AKT and other kinase pathways and by inducing apoptosis. Andrographolide induced both intrinsic and extrinsic apoptosis pathway in different cancer cells via expression of different anti-apoptotic protein like Bax, p53, and activated caspases. Andrographolide was successfully used as an antineoplastic drug in cancer chemotherapy. Andrographolide inhibited the growth of human breast, prostate, and hepatoma tumors. Andrographolide and analogues need to be subjected to further clinical and biomedical studies in cancer chemoprevention. Andrographolide could be potent anticancer agent when used in combination with other chemotherapeutic agents.

2. INTRODUCTION

Andrographis paniculata (Burm.f.) Nees, belonging to the family *Acanthaceae*, has various species with medicinal properties widely used in India, Shri Lanka, China, and other South Asian Countries. *A. paniculata* is commonly known as 'king of bitters', and as *Kalmegh* in India (1). *A. paniculata* has diversity of defense response to microbes and pests via cyanogenesis, phytohormone activation, lignifications of cell wall, modification of secondary metabolites, and a long list of therapeutic usage in Indian and traditional medicine (1). Recently natural products and dietary factor have been associated with cancer prevention and risk (2). *A. paniculata* contains various important biological activities like antibacterial, anti-inflammatory, anti-thrombotic and hepatoprotective. *A. paniculata* has also been used for treating animal diseases, e.g. respiratory infection

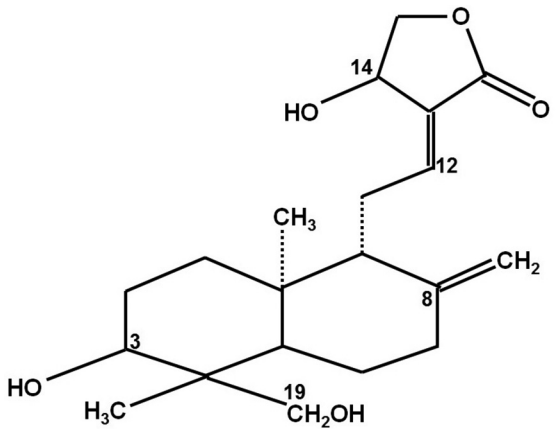

Figure 1. Structure of andrographolide. Andrographolide ($C_{20}H_{30}O_5$) is a labdane diterpenoid, main bioactive component of *Andrographis paniculata*. Andrographolide contains an a-alkylidene c-butyrolactone moiety; two olefin bonds Δ8(17) and Δ 12(13); and three hydroxyls at C-3, C-14, and C-19.

and diarrhoea, as an alternative to antibiotics. Extracts of *A. paniculata* and andrographolide has been widely used *in vitro* and *in vivo* for various pharmacological properties. Ethanolic extract of *A. paniculata* has demonstrated antiviral activity against herpes simplex virus type 1 (3). This plant has been used for the treatment of neoplasm as mentioned in ancient Ayurveda. Adrographolide is the major phytochemical constituent of *A. paniculata*, a bitter most compound among natural products. Pharmacological studies indicate the properties of andrographolide in protection of liver and gallbladder, and have been found to be slightly more active than silymarin (a known hepatoprotective drug) (1). Andrographolide is specifically rated very high in therapeutic action in curing liver disorders, common cough and cold, and inflammation and cancer in humans (1, 4). Andrographolide in general have shown efficiency against allergic reaction, hemorrhagic lesion, central nervous system dysfunction and otheres. In Indian pharmacopoeia, this herb has been formally used as a predominant constituent for various pharmacological preparations as antipyretic and anti-inflammatory (5), hepatoprotective (6), and immunostimulant (7). Derivatives of andrographolide such as neoandrographolide and 14-deoxy-11, 12-didehydro-andrographolide has been utilized in folk medicines for the treatment of not only cancer but also diabetes, kidney disorders, hepatitis and HIV (8). Andrographolide and analogues have been mentioned as ‘‘a novel class of anti-inflammatory and anticancer drugs’’ (9). In this review, we compiled various parameters of this plant with focus on the effects of andrographolide on cancer and the intricate mechanisms involved. We vastly studied and compiled literature to elaborate the effects of andrographolide and derivatives mainly against human cancers. We mainly focused on the role of andrographolide in human cancer and elaborated the cell growth and death mechanisms with future perspectives in cancer prevention.

3. PHYTOCHEMISTRY OF ANDROGRAPHOLIDE AND ANALOGUES

The characteristic secondary metabolites in *A. paniculata* plant have considerably enhanced its importance in the area of phytopharmaceuticals. *A. paniculata* contained several diterpenoids and diterpenoid glycosides with similar carbon skeleton such as andrographolide, neoandrographolide, deoxyandrographolide. Other major phytochemicals from the plant are 14-deoxyandro-grapholide, 14-deoxy-11,12-didehydroandrographolide, andrographiside, deoxyandrographiside, homoandrographolide, andrographan, andrographon, andrographosterin and stigmasterol (1, 4, 5). The basic carbon skeleton of compounds from *A. paniculata* is represented in Figure 1. Andrographolide ($C_{20}H_{30}O_5$) is a colorless, crystalline, highly bitter in taste compound, possessing a lactone function. The structure of andrographolide comprises an a-alkylidene c-butyrolactone moiety, two olefin bonds $\Delta^{8(17)}$ and $\Delta^{12(13)}$, and three hydroxyls at C-3, C-14, and C-19. Of the three hydroxyl groups, the one at C-14 is allylic in nature, and the others at C-3 and C-19 are secondary and primary, respectively (Figure 1). Leaf of the plant contains highest amount of andrographolide (2.3.9%), while the lowest quantity is found in seeds (10). *A. paniculata* is also reported to contain large number of flavonoids and labdane diterpenoids, stigmasterols and xanthones (11, 12). Major labdane diterpenoids of *A. paniculata* are 14-deoxy-11,12-didehydroandrographolide and mostly diterpenes can be isolated in free and glycoside forms. Some flavonoids isolated from *A. paniculata* are 5-ydroxy-7, 8, 6, 2′, 4′-trimethoxyflavone, 5,6-Dihydroxy-7,8-dimethoxyflavone, 5-hydroxy-7,8-dimethoxyflavone (1). Systematic chemical studies of *A. paniculata* have been carried out by various researches during last several years. Structure activity relationships of 19 andrographolide analogues were done, out of which a number of andrographolides showed higher cytotoxic activities than parent compound. 19-O-triphenylmethyl ether

analogue 18 showed higher cytotoxic activity than the potent cancer drug ellipticine (13). The expression analysis of 14-deoxy-11, 12-didehyroandrogrpholide genes showed that it regulates genes of cell cycle and promotes cell cycle arrest in 47D breast carcinoma cells as well as it may cause autophagic morphology in cells (14). Researches in recent past have confirmed that andrographolide has a surprisingly broad range of pharmacological effects; some of them are extremely beneficial. Yet the functional activities of the andrographolide and derivatives remain largely unknown in several human diseases including cancer.

4. ANDROGRAPHOLIDE AGAINST HUMAN CANCERS

Andrographolide and analogues have been generally used against allergic reaction, hemorrhagic lesion, central nervous system dysfunction and others disease. Andrographolide and derivatives are reported to contain high therapeutic potentials against liver disorders, common cough and cold, and inflammation and cancer in humans. These metabolites have been used as/in antipyretic, anti-inflammatory, hepatoprotective, immunostimulant, and anti-neoplasm. The low aqueous solubility of andrographolide causes lower bioavailability subsequently used for oral administration in appropriate tissues localization therefore used in poor therapeutic purpose (15). Andrographolide and derivatives have an excellent property that they do not stay in body for a long time due to short half-life and easy excretion via urine and gastro intestinal tract.

4.1. Hepatoprotection

Andrographolide and derivatives have been long used as hepatoprotective agent curing various types of liver damage and this property has become an important point of attraction in scientific community (1, 16-20). Andrographolide has been identified as major anti-hepatotoxic component of the plant that has exerted profound protective effect *in vivo* against hepatotoxicity induced by CCl_4 (16, 17), D-galactosamine (19), paracetamol, and ethanol (20). Andrographolide was more effective than silymarin, the standard hepatoprotective agent (1). Although poor solubility of andrographolide in water decide limited criteria in clinical field but recently ten andrographolides are designed having aqueous solubility and hepatoprotective activity against carbon tetrachloride (CCl_4)-induced liver injury in mice (17). Various studies have demonstrated that andrographolide is effectively used as a hepatoprotective and hepatostimulative agent against different hepatotoxin. In a study, hexachlorocyclohexane-treated mice administered with andrographolide showed capability to modulate liver function enzymes that catalyze the reactions for several liver damage condition (18). It is proven that andrographolide effectively work against large number of hepatotoxins which might occur due to activation of antioxidant enzymes in liver (19). Andrographolide and analogues of *A. paniculata* have shown anti-hepatotoxic activity *in vitro* and *in vivo*. Intraperitoneal (I.P.) administration of a methanol extract of the aerial parts of the plant to mice (861.3. mg/kg body weight) reduced CCl_4-induced hepatotoxicity by reversing histopathological damages in the liver (16). Also the I.P. administration of andrographolide to mice (100 mg/kg body weight) inhibited the CCl_4-induced increase in the activity of liver function enzymes like serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, alkaline phosphatase, hepatic triglycerides and bilirubin (16). The I.P. administration of a methanol extract of the aerial parts of plant to rats (500 mg/kg body weight) also suppressed the CCl_4-induced increase in the activity of liver function enzymes and bilirubin. Also the intragastric administration of an aqueous extract of the aerial parts of the plant to ethanol-treated rats (500 mg/kg body weight) decreased the activity of liver function enzymes and suppressed histopathological changes in the liver (20). These reports comparatively represent andrographolide as a strong hepatoprotective agent with no known side-effects.

4.2. Antiplatelet aggregation

Platelets play important role in thrombosis, wound healing and hemostasis. Andrographolide and analogues have shown platelet proapoptotic potency. Studies report that andrographolide covalently modified reduced cystein 62 of p50 with the help of underline mechanism NF-κB inactivation in inflammation and neointimal hyperplasia (21, 22). A key component of blood coagulation is tissue factor, whose expression is regulated at the transcriptional level (23). NF-κB is responsible for the regulation of tissue factor expression by working as a principle initiator of coagulation cascade; whereas p50 is highly important to the modulation of tissue factor (23). Direct interaction of p50/p65 hetrodimer with NF-κB is a site of human tissue factor promoter and p50 is essential for the pathogenesis of deep vein thrombosis. Andrographolide was found to work as specific inhibitors of p50, which further caused

reduction of venous thrombosis (24). Fibrionolysis is the ordinary procedure in the body for dissolving blood clots as well as for preventing cardiovascular disease. Researchers reported that an extract of *A. paniculata* produced antihypertensive effects when aqueous extract was injected intravenously to hypertensive rats (25). Andrographolide showed antihypertensive effect because it relaxes the contraction of smooth muscle walls of blood vessels by the inhibition of noradrenaline. Noradrenaline is a hormone responsible for constriction of blood vessels. Thus, it shows the effectiveness for keeping blood flow and oxygen supply. These studies represent a valuable role of andrographolide in preventing and perhaps treating venous thrombosis. It also reflects possibilities that andrographolide may interact with angiogenesis/vasculogenesis signaling pathways in cancer metastasis.

4.3. Anti-inflammation

Inflammation is a key component in occurrence and pathogenesis several diseases such as allergy, asthma, rheumatoid arthritis, inflammatory bowel diseases, and cancer. The importance of andrographolide and analogues in traditional medicine has been enhanced by virtue of their anti-inflammatory properties. Andrographolide decreased the production of chemokine, cytokines and lipid mediators, and inhibited NF-κB signaling pathways. It caused anti-inflammation that might inhibit the PI3K/Akt pathway and downstream target of NF-κB activation in human umbilical vein endothelial cells (HUVECs) (26). Andrographolide has been reported to affect inflammation process *in vitro* by suppressing chemotactic migration of macrophages towards chemo-attractants (27). It suppressed chemotactic migration of macrophages by inhibiting phosphorylation of mitogen-activated protein kinase kinase (MEK) 1/2 and the downstream mitogen activated protein kinase (MAPK) p42⁄p44 (extracellular signal related kinase (ERK) 1⁄2), as well as Akt signaling pathways (27). Andrographolide exhibited anti-inflammatory effect by inhibiting the production of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) in RAW264.7. cells treated with lipopolysaccharide (LPS). This further inhibited NF-κB expression. NF-κB is a transcription factor that enhances cell proliferation and inhibits cellular apoptosis. Andrographolide covalently modified reduced cysteine in oligonucleotide binding pocket of p50 for inhibition of NF-κB activation. This action of andrographolide could be used to treat oral squammous cell carcinoma (28). Andrographolide also suppressed the LPS-induced mRNA expression of suppressor of cytokine signaling (SOCS1 and SOCS3), which in turn inhibited apoptosis signaling and mitochondrial membrane potential activation (29). Macrophage activation is known to induce generation of pro-inflammatory mediators like TNF-α, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-1b, IL-6, IL-8, iNOS, nitric oxide (NO) and COX-2. The production of these cytokines, chemokines, and enzymes were inhibited by andrographolide and isoandrographolide (29, 30). Certain adhesion molecules (such as E-Selectin and ICAM-1) are crucial for the recruitment of inflammatory cells at the site of infection or tissue injury. E-Selectin mediates the recruitment and initial attachment of leucocytes (such as neutrophils), certain subsets of T lymphocytes and eosinophils (29). Andrographolide can downregulate TNF-α-induced expression of ICAM-1 and E-selectin leading to inhibition of endothelial-monocyte adhesion (31, 32). Andrographolide has been reported to suppress N-formylmethionyl-leucyl-phenylalanine induced neutrophil adhesion and activation by inhibiting macrophage-1 antigen expression (33). Upregulation of Mac-1 together with IL-2 and TNF-α induces apoptosis and selective removal of the cell and may cause a prolongation of the life of the immune cell. This resulted in subsequent reduction of protein kinase C-dependent production of reactive oxygen species (ROS) and NO (33). Also andrographolide caused inhibition of expressions of iNOS mRNA and protein in macropahges (34). High levels of iNOS and NO is known to cause tissue damage because of strong oxidative effects and interactions with superoxide anion (O^{-2}) to generate peroxynitrite ($ONOO^{-}$), which causes stronger oxidative insult than NO (35). Thus, inhibition iNOS expression and reduced production of NO is considered a critical therapeutic action of andrographolide. Andrographolide has also been shown to effectively stimulate the immune system mainly via antigen-specific response and nonspecific immune response. Andrographolide enhanced immune responses through production of lymphocytes, activation of lymph system (perform as a shuttle for invading bacterial and viral), release of interferon/cytokine (a strong antiviral agent). Andrographolide activates both responses making it effective against a variety of infectious as well as oncogenic agents (7). These properties of andrographolide may connect to anti-inflammation through specific cellular signaling mechanism. Collectively, these reports emphasize the anti-inflammatory potentials of andrographolide through specific mechanism in various cellular conditions.

4.4. Anticancer

Cancer is a leading cause of death in developed nations and spreading fast in other countries. Cancer is resultant of self-sufficiency in proliferative growth signals, insensitivity to growth inhibitory signals, evasion of apoptosis, induction of angiogenesis, induction of invasion and metastasis. Tumor microenvironment surrounding the malignant cells regulates the development and progression of tumors and metastasis (36). Tumor microenvironment in general is inflammatory in nature with high redox activities offering new prospects for therapeutic interventions. Natural products have been considered as primary source for providing chemotherapeutic agents with promising antitumor activities (37). In recent years, anticancer drug development strategies targeted not only classical pathways but shifted to targeting the specific signaling pathways like Wnt/β-catenin, Hedgehog, Hippo, NF-κB, STAT, p53, and PI3-K/AKT/ERK (38). We have conducted several studies modulating these pathways using natural and synthetic agents and evidences suggest that the combination of phytochemicals with synthetic agents may serve as an effective anticancer therapeutic strategy (39-42). Thus a recent interest exists in identifying anticancer agents from medicinal herbs as new and innovative drugs with lesser side-effects. Andrographolide and analogues have shown inhibitory properties against proliferation of different cancer cell lines such as leukemia, breast, lung, and melanoma (4, 43, 44). Andrographolide showed versatile properties by exhibiting the inactivation of AKT and ERK signaling, and inhibition of matrix metalloproteinase 2 (MMP2) activity. Consequently it suppressed the invasion ability in colon cancer cell (CT26 cell) (45). MMPs and its regulatory pathways have been bright chances for anticancer drugs research. A recent study demonstrated that andrographolide controls the regulatory pathways by affecting several genes dominantly involved in apoptosis, cell cycle and adhesion related biological signaling including MAPK, focal adhesion and tight junction pathway (46). This suggests that andrographolide could be new anticancer agent in treatment of NSCL. Another study gave an assumption that andrographolide affect the downregulation of PI3K/AKT signaling and suppression of c-Jun/c-Fos (AP-1 hetrodimer complex). It is responsible for inhibition of cell migration or invasion and consequently suppression of MMP-7 expression (47). Furthermore, it suppressed both MMP-2 and MMP-9 activity (essential in angiogenesis) in HUVECs (45). An interesting study deduced the molecular basis of andrographolide-mediated anticancer activity through a novel mechanism involving inhibition of Hsp90 function and reducing the levels of Hsp90 client proteins (48). Andrographolide and derivative regulated the CYP1A super family gene, a gene generally expressed in several extra hepatic tissues responsible for the activation of heterocyclic amine and carcinogenic amino acids. Andrographolide acted as an antagonist ligand on aryl hydrocarbon receptor expression (49). Andrographolide and analogues have shown promising anticancer activities and their mechanisms may vary depending on the types of cancer cell *in vitro* and *in vivo*.

4.4.1. Cytotoxicity

The cytotoxic effect of andrographolide and analogues depend on concentration of dose and time of treatment in cancer or non-cancer cell lines. Cytotoxicity in general means toxic to cells but cancer cell specific toxicity is in current demand. Andrographolide was found to have cytotoxic effects on human hepatoma cell lines HepG2 but no any effect in normal liver L-02 cells (50, 51). A study also showed that andrographolide and derivatives enhanced the autophagy markers in various cancer cell lines and likely to suppress autophagic flux at maturation and degradation stage (52). Andrographolide showed significant cytotoxic activity against KB (human epidermoid leukemia) and P388 (lymphocytic leukemia) cell lines. Exploring the cytotoxic properties of andrographolide on human liver cancer cell showed that andrographolide induced autophagic cell death by disrupting mitochondrial membrane potential and elevation of reactive oxygen species (ROS). The autophagy caused by andrographolide is deduced by observing accumulation of LC3-II protein as well as autophagosomes and formation of puncta GFP LC3 (32). One another andrographolide conjugate (andrographolide-lipoic acid) also exerted anticancer cytotoxicity by the induction of apoptosis in human leukemia K562 cells through the ROS-dependent DNA damage (53). A recent study demonstrated the antiproliferation and cytotoxic effects of andrographolide (54). Andrographolide can penetrate blood brain barrier and concentrates in brain. Thus, its effect studied on glioblastoma showed that it induces cell cycle arrest at G2/M phase along with downregulates cdk 1 and cdc25 proteins. Andrographolide caused cytotoxicity in glioblastoma cells causing decrease in the activity of PI3/Akt signaling, reduced expression of PI3K and pAkt, pmTOR (54). The 14-deoxyandrographolide from an ethanolic extract of *A. paniculata* showed four fold high cytotoxic effect as compared with water

extract (55). In a study of andrographolide analogues, most of the compounds exhibited significant cytotoxicity as compared to andrographolide. The underline mechanism observed was that C-19-hydroxyl group of andrographolide oxidized as a carboxyl group and subsequently esterified to form carboxylic acid, leading to the novel cytotoxicity against cancer cells (56).

4.4.2. Apoptosis induction

Apoptosis is programmed cell death, a signaling event mediated by activation of an evolutionary conserved intracellular pathway. Recent trends show firm relationship between apoptosis and cancer mediating cancer progression and metastasis. Cancer cells are characterized as slow apoptotic in nature. Recently various natural products as well as synthetic anticancer agents targeted to reactivate apoptotic cascades and to make cells apoptosis prone so that low doses of cytotoxic drugs can cause apoptosis (40-42, 57). Andrographolide and analogues have been reported to induce apoptosis in various cancerous conditions. Andrographolide was responsible for the activation of caspas-3 and p53, furthermore it inhibited NF-κB activity, and induced cell death in human neuroblastoma cells (58). NF-κB is a major transcription factor, which controls cell apoptosis and proliferation (59). A combination of andrographolide with fluorouracil (5-FU) enhanced apoptosis in human hepatocellular carcinoma (HCC) cell line SMMC-7721 and suppressed the p53. It resulted in altered Bax conformation and activations of caspase-3,8,9, disruption of the mitochondrial membrane potential, increased release of cytochrome c in the process of apoptosis reactivation (60). In a study (61), andrographolide was presented for potential use in cancer therapy. Andrographolide was found responsible for p53 phosphorylation and activated p53 induced transcriptional up-regulation of death receptor 4 (DR4). This activation led to the apoptosis activation through tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). Andrographolide enhanced DR4-mediated TRAIL-induced apoptosis in TRAIL-resistant cells (61). The treatment of andrographolide to T-47D mammary cells caused internalization of epidermal growth factor receptor (EGFR) and transferring receptors (TfRs) in to cell due to downregulation on cell surface receptors and degradation of EGFRs and TfRs (62). Andrographolide inhibited IL6 expression (required for proliferation of prostate cancer) at both mRNA and protein and induced apoptotic cell death. Thereby andrographolide could be represented as a therapeutic agent to treat both androgen stimulated and castration resistant prostate cancer (63). DNA topoisomerase II modification is among other novel mechanisms and a hopeful chemotherapeutic target for anticancer agents against different type of cancer. An andrographolide analogue inhibited DNA topoisomerase II α at a low concentration and induced apoptosis in cholangiocarcinoma (64). Andrographolide induced apoptosis in different cancer cell lines and led to the inhibition of cancer cell growth yet it remains unclear that which receptor-trafficking in cells is majorly affected by andrographolide.

4.4.3. Cell cycle arrest

Andrographis and its compounds have been found to counteract with the cell cycle progression in cells, such interference is the remarkable point of the development of anticancer strategies. Andrographolide affected the human breast cancer cell and attenuated endothelial cell motility and tumor-endothelial cell interaction. It suppressed the tumor growth by arresting cell cycle at G2/M phase and induced apoptosis through caspase independent pathway (65). Similar reports by another experiment that andrographolide inhibited the growth of human hepatoma cells by arresting at G2/M phase of cell cycle and inducing late apoptosis. Andrographolide caused reduction of glutathione stimulating hormone (GSH) concentration and increase of hydroxyl peroxide (H_2O_2) production in mitochondria as well as also decrease in super oxide radicals (50). Andrographolide has been anti-proliferation effect in colorectal cancer cells by inhibiting cell cycle related protein through enhancing the expression of cell cycle inhibitory protein - p16, p21, p53 (66). It consequently decreased the levels of cell cycle regulatory proteins cyclin A, Cyclin D, CDK4, CDK2, those required for G1-S transition and further inhibited Rb phosphorylation. Most of human colorectal carcinoma Lovo cells were arrested in G1 phase after treatment of andrographolide via induction of p27 and the suppression of CDK4 in human tumour cell lines (66). Some andrographolide analogue like 3A.1 19-tert-butyldiphenylsilyl-8, 17-epoxy andrographolide showed caspase-3 activation and reduction in the expression of CDK6, cyclin D1, and COX-2 proteins. It also suppressed the expression of DNA topoisomerase II α. This is found capable for induction of apoptosis in primary malignant tumor of bile duct epithelial cells (67).

4.4.4. Antitumor

Andrographolide and analogues have been attempted to use in cancer chemoprevention

by virtue of their specific anticancer mechanisms. Andrographolide was successfully used as an antineoplastic drug in cancer chemotherapy with minimum side-effect on non-cancer cell. Non-toxic and delivery-efficient andrographolide nanoparticles prepared as PLGA-nanoparticulation of andrographolide with additional chitosan coating showed increased anticancer efficacy in human breast cancer cells and Ehrlich ascites carcinoma mouse model (68). Different andrographolide showed improved chemotherapeutic efficiency in nanoparticle for with enhanced anticancer property through G1 phase cell cycle arrest and induction of apoptosis in breast cancer MCF7 cell line. The same was proved by *in vivo* experiment, in which andrographolide-nanoparticle reduced tumor weight more efficiently as compared to androrapholide (68). Andrographolide-nanoparticle appeared to be a safe anticancer preparation with enhanced efficacy. Some andrographolide treatment has shown to downregulate PI3K/AKT signaling pathway consequently decreasing the expression of hypoxia inducible factor-1α (HIF-1α) by the ubiquitin-dependent degradation (69). HIF-1α is responsible for tumor growth in non-small cell lung cancer (NSCLC) and A549 cell. Andrographolide treatment inactivated HIF-1α, decreased the level of VEGF, and increased the expressions of hydroxyl-HIF-1α and prolyl hydroxylase (69). This highlight the potentials of andrographolide for development as chemotherapeutic or anti-angiogenesis agent for treatment of NSCLC in the future. A study reported the protective effect of andrographolide against cyclophosphamide (CTX)-induced urothelial toxicity (70). Pretreatment of Swiss albino mice with andrographolide and *A. paniculata* extract could significantly reduce CTX-induced urothelial toxicity. Andrographolide inhibited the production of pro-inflammatory cytokine (TNF-α), which was elevated during CTX administration. In addition, andrographolide elevated the levels of IL-2 and IFN-γ that were lowered by CTX treatment (70). Andrographolide may act as ligand to inhibit GDP-GTP exchange by binding to transient pockets of Kirsten-Ras (K-Ras). In consequence, it reduced the GTP loading of wild type K-Ras in response to acute EGF stimulation. On prolonged treatment of andrographolide, it also reduced signal transmission by oncogenic mutant K-RasG12V by binding to Ras (71). This demonstrated that it is a valid approach to abrogate the function of oncogenic mutant Ras. One of the derivatives of andrographolide, dehydroandrographolide, was reported as a new anticancer drug having good absorption and metabolism stability in intestine. Dehydroandrographolide have good absorption rate in intestine but there is no any significant reaction observed in intestinal perfusates. There are various amino analogues (β-amino-γ-butyrolactone) synthesized from andrographolide having high stereo selectivity with good yield. These amino analogues exhibited cytotoxic properties against six cancer cell lines, revealing that such analogues could prove to be good anticancer agents (72). The treatment of andrographolide in combination with cisplatin or doxorubicin increased cytotoxicity in neuroblastoma (58). These reports highlight the antitumor properties of andrographolide and analogues, alone or in combination, with potentials to develop new therapeutic strategies.

5. CONCLUSIONS AND FUTURE DIRECTIONS

Natural products have shown significant contributions in anticancer therapies. Several potent and effective anticancer agents, such as aspirin, vincristine, vinblastine and paclitaxel, are derivatives of plant-derived bioactive molecules. *Andrographis paniculata* has been used in for medicinal purposes in traditional medicine in several countries including India. Andrographolide is among main bioactive molecule having immunosuppressive, antipyretic, analgesic, hepatoprotective, antiviral and antiinflammatory properties. The cumulative effects and mechanism of action of andrographolide has been represented in Figure 2. Andrographolide and analogues induced apoptosis in various cancer cells and caused cell cycle arrest, and showed antitumor properties. Andrographolide and analogues induced cell cycle arrest and apoptosis, inhibited metastasis and anti-angiogenesis in both animal and human cancer cells. The mechanisms behind effects of andrographilode was broadly through inhibition of v-Src, NF-κB, STAT3 and PI3K/AKT activity and downregulation of mediators of cell cycle progression, inflammation, metastasis and angiogenesis. Andrographolide and analogues have been subjected to extensive chemico-biological investigations for anticancer drug development. Several andrographolide analogues have shown superior anticancer activities in both *in vitro* and *in vivo* models. Further clinical and biomedical studies are required to confirm the pharmacological, pharmaceutical and toxicological properties of andrographolide. In addition,

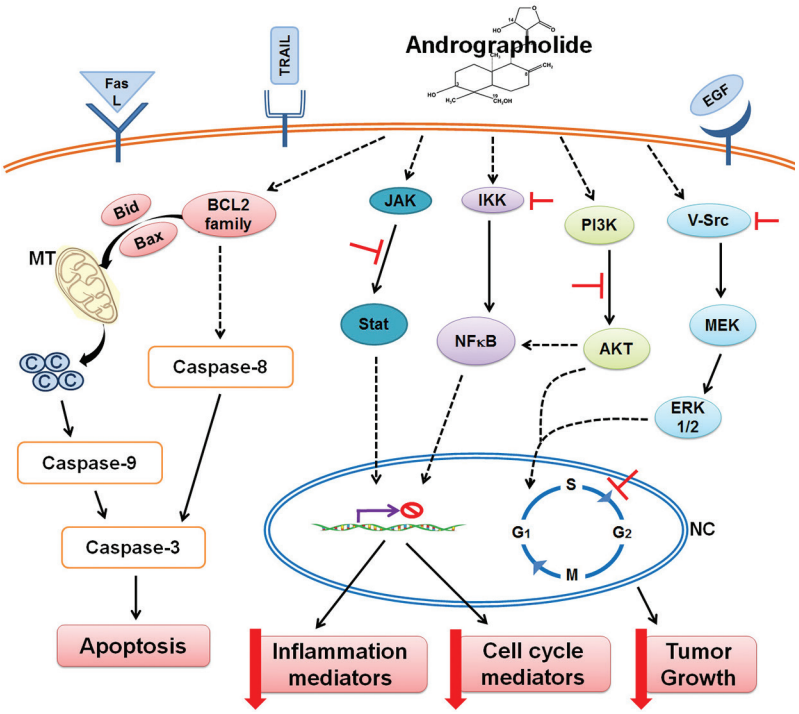

Figure 2. Schematic representation of the mechanism of effects of andrographolide in cancer. Andrographolide interact with several receptors binding sites at cell membrane and transduce respective signaling events leading to various phenomenon such as apoptosis induction, inhibition of inflammation, cell cycle arrest, and tumor growth inhibition. MT, mitochondria; NC, nucleus.

combined drug discovery and combinatorial studies with andrographolide analogues may serve helpful in cancer therapeutics.

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