Abstract: Anthraquinones represent a large family of compounds having diverse biological properties. Emodin (1,3,8-trihydroxy-6-methylanthraquinone) is a naturally occurring anthraquinone present in the roots and barks of numerous plants, molds, and lichens, and an active ingredient of various Chinese herbs. Earlier studies have documented mutagenic/genotoxic effects of emodin, mainly in bacterial system. Emodin, first assigned to be a specific inhibitor of the protein tyrosine kinase p65/ck, has now a number of cellular targets interacting with it. Its inhibitory effect on mammalian cell cycle modulation in specific oncogene overexpressed cells formed the basis of using this compound as an anticancer agent. Identification of apoptosis as a mechanism of elimination of cells treated with cytotoxic agents initiated new studies deciphering the mechanism of apoptosis induced by emodin. At present, its role in combination chemotherapy with standard drugs to reduce toxicity and to enhance efficacy is pursued vigorously. Its additional inhibitory effects on angiogenic and metastasis regulatory processes make emodin a sensible candidate as a specific blocker of tumor-associated events. Additionally, because of its quinone structure, emodin may interfere with electron transport process and in altering cellular redox status, which may account for its cytotoxic properties in different systems. However, there is no documentation available which reviews the biological activities of emodin, in particular, its growth inhibitory effects. This review is an attempt to analyze the biological properties of emodin, a molecule offering a broad therapeutic window, which in future may become a member of anticancer armamentarium.

Key words: emodin; apoptosis; antitumor; reactive oxygen species; chemotherapy
1. INTRODUCTION

Extracts from the roots, bark, or dried leaves of senna, cascara, aloe, frangula, rhubarb, and buckthorn have been used as laxatives since ancient times and are currently used in the preparation of herbal laxatives. The common ingredients include anthraquinones, dianthraquinones, sennosides, naphthalins, stilbenes, tannins, flavonoids, and several polyphenols. Anthraquinones are mostly present as their glycoside derivatives. Anthraquinone glycones are poorly absorbed from the gastrointestinal tract but are cleaved by the gut bacteria to produce aglycones that are easily absorbed and are considered responsible for the purgative properties of these preparations. Emodin (1,3,8-trihydroxy-6-methylanthraquinone) (Fig. 1) is a naturally occurring anthraquinone present in the roots and barks of numerous plants and an active ingredient of Chinese herbs including *Rheum officinale* and *Polygonum cuspidatum*.1–3 Emodin is also produced as a secondary metabolite by molds and lichens; it was known as a diarrheagenic toxin from culture extracts of *Aspergillus wentii* Wehmer isolated from weevil-damaged Chinese chestnuts.4,5 Pharmacological studies using crude as well as pure forms of emodin have been carried out so far and has indicated emodin’s role as a purgative, antibacterial, immunosuppressive, vasorelaxant, cardiotonic, hepatoprotective, and anticancer in nature.6–12 *Polygonum cuspidatum* (which contains emodin) has also been traditionally used for menoxenia, skin burn, gallstone, hepatitis, inflammation, and osteomyelitis in China.13 The crude drug is also reported to be useful for the treatments of hemorrhage of the digestive system and extermination of *Helicobacter pylori*.14–16 The dried roots of *Rumex patientia* L (contains emodin) have been used as purgative, depurative, and tonic in Turkish traditional medicine.17 Even though there are a variety of compounds present in these crude extracts, emodin happens to be a principal component responsible for the biological properties associated with it. Elaborate research has been done towards looking into the mutagenic/carcinogenic potential of this compound. Recent research, however, has concentrated on deciphering growth inhibitory activities of emodin in specific growth factor overexpressed cells and the apoptosis-inducing capacity of emodin either alone or in combination with other chemotherapeutic drugs. Its additional effect on angiogenic and metastatic processes makes emodin a molecule with a broad therapeutic value. It is very apparent that emodin has placed itself as a putative antitumor agent due to elaborate molecular studies done in the recent past. This review briefly describes the work done so far in this field and also an update on the mechanism of emodin action in mammalian cells.

2. EMODIN AS LAXATIVE

Emodin (with other anthraquinones and their derivatives) forms the basis of a range of natural purgatives and is used as a laxative since ancient times, eventhough the actual mechanism of action was not fully understood. Anthraquinone-based cathartics are believed to increase the rate of contraction of intestinal tissue *in vitro* via multiple mechanisms. A tentative model in this regard is depicted in Figure 2. It is now understood that emodin exerts its laxative property due to its chemical

![Figure 1. Structure of emodin.](image-url)
and biological characteristics. It is believed that the presence of hydroxyl groups in position 1 and 8 of the aromatic ring system is essential for the purgative action of the compound.\textsuperscript{18} Because of its chemical structure, emodin glycoside (and other anthraquinones too) in mammals is carried unabsorbed to the large intestine, where metabolism to the active aglycones takes place by the intestinal bacterial flora. The released aglycone exerts its laxative effect by disturbing epithelial cells, which leads directly and indirectly to changes in absorption, secretion, and motility.\textsuperscript{19} Recent viewpoints hold that emodin can enhance the excitability of smooth muscles of the intestine. In support of the above assumption, emodin was found to enhance the function of small intestinal peristalsis in mice, evidenced by increased charcoal powder propelling ratio of small intestine.\textsuperscript{20} The possible mechanism was found to be through the enhancement of motilin (a hormone secreted by the
small intestine that increases gastrointestinal motility) by emodin. In another study, emodin caused contraction of the ileum by triggering the release of endogenous acetyl choline, which acts on muscarinic receptors to cause contraction of the rat isolated ileum preparation. Emodin could directly contract the colonic smooth muscle in multiple organ dysfunction syndrome (MODS) model rats, mediated by activation of myosin light chain kinase (MLCK) by calcium ion release or by stimulating protein kinase C-alpha (PKC-α) pathway for increased calcium sensibility. Further, emodin could inhibit the secretion of somatostatin, a hormone inhibiting gastrointestinal function. Emodin could also increase fluid electrolyte accumulation in the distal ileum and colon (change in absorption and secretion of water; retention of potassium) through unknown actions, possibly via an irritation of the intestinal mucosa and endothelial cells. Activity of Na\(^+\)-K\(^+\)-ATPase in intestinal mucosa was decreased by emodin, which resulted in the decreased absorption of glucose, aminoacids, and sodium ions by intestinal tract resulting in the enhancement of osmotic pressure in the enteric cavity leading to augmentation of peristaltic activity.

Alternatively, emodin is thought to act through inhibiting the activity of K\(_{\text{ATP}}\) channel and the ion transport (chloride) across colon cells, contributing to the laxative effect. There is also a report showing increased prostaglandin synthesis by the intestinal tissue when exposed to aloin and 1,8 dioxyanthraquinone.

In summary, emodin glycosides may be cleaved by the intestinal bacteria to release emodin, which acts either directly or indirectly on colon epithelial cells. This in turn activates the underlying smooth muscle cells leading to muscle contractility. However, chronic use can cause disturbance of electrolyte balance, especially potassium deficiency, and fluid imbalance. Pigment implantation into the intestinal mucosa (Pseudomelanosis coli) is harmless and usually reverses on discontinuation of the drug. The hydroxyl groups present in the aromatic rings permit emodin to interact with proteins by forming hydrogen and ionic bonds, and it partially explains the various interactions of emodin with enzymes, transporters, channels, and receptors. Thus emodin gains access to multiple targets and thus becomes multifunctional compound.

3. MUTAGENICITY OF EMODIN, IS THERE?

Reports that 1,8-dihydroxyanthraquinone, a commonly used laxative ingredient, caused tumors in the gastrointestinal tract of rats raised the possibility of an association between colorectal cancer and the use of laxatives containing anthraquinones. Because emodin is structurally similar to 1,8-dihydroxyanthraquinone and is present in herbal laxatives, many studies have been done for testing its mutagenic potential, mostly using prokaryotic systems. Emodin isolated from different sources was reported to be mutagenic in Salmonella typhimurium strains TA97, TA98, TA100, TA102, and TA1537 with or without metabolic activation. Emodin is biotransformed by the microsomal cytochrome P450 enzymes into hydroxyemodins, some of which are direct mutagens to the test strains and could, therefore, explain the basis for mutagenic nature of emodin. Alternatively, 2-hydroxyemodin, one of the metabolic products of emodin, in turn can produce active oxygen and can induce DNA strand breaks suggesting a possible role of active oxygen in the process of mutagenesis, even though there is a report against the involvement of oxygen. Another conceivable mechanism could be the non-covalent binding of emodin to DNA leading to the inhibition of the catalytic activity of topoisomerase II, at least in part, contributing to emodin-induced genotoxicity and mutagenicity. Spore rec-assay with a recombination-deficient mutant of Bacillus subtilis M45 demonstrated the DNA damage-inducing activity of emodin. Under Multiple Computer Automated Structure Evaluation (MULTICASE) system for evaluation of carcinogenicity, emodin was predicted to be a rodent carcinogen. Based on its chemical characteristics and its varied biological functions, and its possible mutagenicity, emodin has been evaluated prospectively for carcinogenicity and overt toxicity by Computer Optimized Molecular Parametric Analysis for
Chemical Toxicity (COMPACT). This overall prediction has been made on the basis of the results of the computer tests and from consideration of the information from bacterial mutagenicity, together with likely lipid solubility and pathways of metabolism and elimination. Emodin was predicted positive for carcinogenicity. To obtain an in depth knowledge regarding its predicted mutagenicity and to verify its historical claims of potential benefits, 2-year genetic toxicology and carcinogenesis studies of emodin were conducted by National Toxicological Program (NTP) of National Cancer Institute (NCI), USA. The results showed no evidence of carcinogenic activity for emodin in male F344/N rats and female B6C3F1 mice and equivocal evidence of carcinogenic activity in female 344/N rats and male B6C3F1 mice. Therefore, assessment of the genotoxicity profile of emodin in light of other data from animal metabolism and rodent carcinogenicity studies do not support concerns that senna laxative components pose a genotoxic risk to humans when consumed under prescribed use conditions.

However, there are also reports showing no evidence of mutagenicity for emodin. In mammalian test systems using V79 Chinese hamster cells, no genotoxicity of emodin was found either with or without metabolic activation. Lack of emodin genotoxicity was also evident in a mouse micronucleus assay.

There are reports about antimutagenicity of emodin in Salmonella typhimurium TA98. The crude extracts (containing 3.4 mg of emodin, 2.1 mg of chrysophanol, and 1.8 mg of rhein in 10 g of dry matter) as well as emodin induced a dose-dependent decrease in the mutagenicity of benzo[a]pyrene (B[a]P), 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2), and 1-Nitropyrene. Most procarcinogens require metabolic activation to its active form by cellular detoxifying enzymes. Emodin has been shown to inhibit cytochrome P4501A1, thus countering the effects of mutagen. Among a panel of 10 anthraquinones, emodin emerged as the most active inhibitor for cytochrome P4501A1. The reason for the varied effects as shown above by emodin is not clear. Overall, there is no consensus regarding the presence of mutagenicity for emodin by the several in vitro/vivo assays reported. On the whole, the mutagenicity of emodin may depend on (i) its activation by microsomal enzymes, (ii) the cell system in which these studies are carried out. It seems when used alone, emodin exhibits mild mutagenicity at least in microbes.

4. EMODIN AS ANTIMICROBIAL AGENT

Because of its capacity to interfere with cellular metabolism, emodin has shown generalized antimicrobial effects against select microorganisms. Inhibition of growth study from H. pylori demonstrated that emodin elicited a dose-dependent growth inhibition in H. pylori cultures. Antibacterial effects on Escherichia coli K12, Pseudomonas aeruginosa PAO1, and some strains of Staphylococcus aureus by emodin were also documented. Emodin was also found to possess virucidal activity and fungicidal activity. Emodin can cause inhibition of electron flow in the respiratory chain, most likely in between ubiquinone and cytochrome b, and also cause dissipation of the proton motive force. Emodin can also be reduced to its semiquinone, and in presence of oxygen, generation of superoxide can occur. These three effects on energy transduction in cytoplasmic membranes may explain the antibiotic properties of emodin.

5. ANTI-INFLAMMATORY NATURE OF EMODIN

Emodin has shown anti-inflammatory effects in various experimental models, and any molecules falling in this category are generally considered augmenting cancer therapy. Emodin’s anti-inflammatory effect was first identified on carrageenan-induced edema in rat models. However,
the mechanism of anti-inflammatory effects was unknown and there are different explanations for the observed activity. It might be due to the inhibition of nitric oxide (NO) production, or through impairment of cytokine production, or through reduction of prostaglandin synthesis, or even by its inhibitory effect against superoxide production. However, the most acceptable mode of anti-inflammatory action of emodin is due to its specific inhibition of NF-κB, an important transcription factor.

6. EMODIN AS A REDOX REGULATOR

Emodin (or emodin containing extracts) seems to accomplish an antioxidant status due to different mechanisms such as inhibition of radical formation, radical scavenging, inhibition of lipid peroxidation, and enhancement of antioxidant defences. It is clear from the above studies that emodin behaves as a protector of cell constituents in presence of an oxidant stress. In most of the above studies, the cells/cell constituents were challenged by an oxidant and emodin’s role in negating the effect was documented. However, recent studies using emodin in cancer cells convey a different viewpoint (that emodin being a prooxidant) and are presently holding an upperhand. It is appropriate to speculate the expansion-reactive oxygen species (ROS) generation by emodin, considering the structural characteristics of emodin. It being a quinone and an effective electron acceptor is likely that emodin intracellularly interacts with molecular oxygen and generates superoxide anion. There are a number of recent studies showing emodin’s ability to generate ROS in a variety of tumor cells. The ROS thus produced may suppress the important transcription factors needed for cell survival and may also degrade key proteins and nucleic acids within the cancer cells. However, reason for the earlier report of emodin not generating ROS in HL-60 leukemic cells is not clear. But it is a known fact that most pro/antioxidants behave differently depending on the experimental conditions and is possible that such phenomenon works true for emodin too. This may also reflect more when cell lines of different tissue origin are used for analysis; it is known that cells vary with respect to their antioxidant status.

7. CELL CYCLE INHIBITION BY EMODIN

Emodin suppressed the proliferation of renal tubular cells in a dose dependent manner, as measured by the uptake of radiolabeled thymidine. Further, it selectively blocked the growth of v-ras-transformed human bronchial epithelial cells with little effect on the growth of normal human bronchial epithelial cells. This study led to the concept that emodin may interact with targets involving oncogene signaling pathways. Emodin is now known to be moderately cytotoxic and have growth inhibitory properties in many cell types. Antiproliferative activity of emodin was reported to be as a result of its effects on interfering with the progress of cell cycle in a variety of cells including human fibroblasts, smooth muscle cells, endothelial cells, and malignant cells even though the exact mechanism of action of emodin was quite unclear. Emodin was found to decrease the respiratory control index and P/O ratio (the relationship between ATP synthesis and oxygen consumption) in rat liver mitochondria, indicating an uncoupling mode of action. In another study in E.coli, emodin was shown to inhibit electron transfer and uncoupling effects, the site of action being at the level of the natural quinone ubiquinone. Emodin exhibited growth-suppressing effect on HepG2/C3A, PLC/PRF/5, and SK-HEP-1 hepatoma cells, by the sub-G1 accumulation, G2/M phase arrest. Thus, emodin displays effective inhibitory effects on the growth of various human hepatoma cell lines and stimulates the expression of p53 and p21 that resulted in the cell cycle arrest of HepG2/C3A cells at G2/M phase.

However in a rat model, a growth-promoting activity of emodin in liver regeneration was reported recently. In another study, induction of DNA synthesis and protein by emodin was
documented possibly through the Transforming Growth Factor (TGF)-β1 gene expression enhancing pancreatic repairing and remodeling. It is speculated that emodin does so by enhancing cytokine TGF-β1 gene expression, regulating cell growth and differentiation, stimulating the formation of extracellular matrix components. Nonetheless, intervention of cell cycle by emodin led to the idea of using this compound against tumor cells, which gave emodin a new title as an anticancer agent.

8. ANTITUMOR EMODIN—UPGRADATION FROM A MUTAGEN

The earliest reports of emodin as cytotoxic agent in P-388 lymphocytic leukemia and murine leukemia L1210 culture cells came in 1976 and 1984, respectively. Later, emodin was found cytotoxic to FM3A, a mouse mammary carcinoma cell line, in concentrations of 1–10 μg/mL. In another report, emodin showed cytotoxic activities against human oral squamous cell carcinoma (HSC-2) and salivary gland tumor (HSG) cell lines than against normal human gingival fibroblasts (HGF). Cell viability test indicated that inhibitory effect of emodin on various tumor cell lines was not through direct cytotoxicity. Several mechanisms have been described as the possible modes of antitumor action of emodin. Inhibition of electron transport chain and uncoupling effects were documented in rat mitochondria. Since anthraquinones are efficient electron acceptors, the possible site of action could be at the site of ubiquinone. The presence of the hydroxyl group in the 1,4 or 1,8 positions may lead to strong intramolecular hydrogen bonding. Electron transfer from the semiquinone to molecular oxygen leads to the generation of the superoxide anion (O2•−) from which a variety of ROS may be generated. Alternatively, alkylation of DNA or other cell constituents has also been thought to be the primary lesion(s) leading to perturbation of the cell cycle. Later, emodin was discovered as a strong inhibitor of a protein tyrosine kinase (p56lck), which was the first report relating emodin to a specific cellular target. Therefore, further studies were initiated to evaluate whether emodin caused inhibition of other tyrosine kinase receptors as well. Emodin was found to decrease tyrosine-phosphorylated proteins in v-ras-transformed human bronchial epithelial cells and exhibited selective cytotoxicity against cancer cells with an activated ras oncogene. Emodin inhibited HER-2/neu tyrosine kinase activity and preferentially suppressed growth and induced differentiation of HER-2/neu-overexpressing breast and non-small cell lung cancer cells and suppressed the growth of HER-2/neu-overexpressing breast cancer cells in athymic mice and sensitized these cells to paclitaxel. These studies clearly revealed emodin’s specific activity in oncogene-stimulated cells of different origins as well as its utility of being used as a sensitizer of antitumor drug therapy. Recently, emodin was also found to block phosphorylation of HER2/neu by heregulin and block the ERK phosphorylation of PC3 prostate cancer cells. There is also a report of emodin causing an increase in PTEN phosphorylation followed by decreased phosphorylation of CK2/Akt/cyclic AMP response element-binding protein (CREB) in the SK-N-SH cells (neuroblastoma cells). Emodin and aloe-emodin, members of the anthraquinone family, inhibited proliferation of the adherent variant cell line of Merkel cell carcinoma. However, the reason for inhibition of proliferation in these cells remains to be found out.

Through a COP9 signalosome (CSN) associated kinases (of which CK2 is a member) dependent mechanism, emodin significantly induced ubiquitination and proteasome-dependent degradation of transiently expressed Id3 in HeLa cells. Emodin enters the nucleotide-binding site of the CK2, filling a hydrophobic pocket between the N-terminal and the C-terminal lobes, in the proximity of the site occupied by the base rings of the natural co-substrates. Emodin has recently been shown to inhibit CK2, which has been identified to regulate angiogenesis. This is probably through CK2’s role in pathways activated by angiogenic pathways including Ras-Raf, PKC, PI3 kinase-Akt, and p38MAPK.

However, using primary cells from an ovarian carcinoma that overexpressed both c-erbB-2 and topoisomerase I-α, the tyrosine kinase inhibitor emodin exhibited no chemosensitizing effect in these
cells of this individual carcinoma. Emodin did not show any effect on human megakaryoblastic leukemia CMK-7 cells. The reasons for the above findings are yet to be investigated.

Yet another mechanism of action of emodin is through its metabolic activation by the microsomal enzymes. It is known to induce cytochrome P450s 1A1 in a variety of cell lines including human lung carcinoma NCI-H322, breast cancer cells, MCF-7, HepG2 hepatoma cells, and human lung adenocarcinoma CL5 cells. Modulation of cytochrome P450 by emodin may be an important factor affecting metabolism and toxicity of the hydroxyanthraquinone in humans. This forms a mode of action in microbial system too. Emodin effectively decreased tumor necrosis factor (TNF) α/-12-O-tetradecanoylphorbol 13 acetate (TPA)-induced promoter activity of GSTP1-1 gene expression in K562 and U937 leukemia cells.

Being an anthraquinone, emodin has shown to behave as a phytoestrogen involving steroid receptor-signaling pathway. Emodin showed potent estrogen receptor binding affinity and enhanced the proliferation of MCF-7 cells. Conversely, emodin treatment resulted in repressing androgen-dependent transactivation of androgen receptor (AR) through heat shock protein (HSP) 90-MDM2 pathways and by inducing AR degradation through proteasome-mediated pathway in a ligand-independent manner.

There are also a few reports of emodin being a chemopreventive agent in experimental studies. Inhibitory effects of water extracts of Cassia tora (contains emodin) on benzo[a]pyrene-mediated DNA damage toward HepG2 cells was documented. Emodin exhibited potent inhibitory activity on two-stage carcinogenesis test of mouse skin tumors induced by NO donor, (+−)(E)-methyl-2-[(E)-hydroxyimino]-5-nitro-6-methoxy-3-hexeneamide as an initiator and TPA as a promoter. Activation of DNA repair machinery may be the mechanism by which emodin exhibits its chemopreventive nature as observed in Reference.

In summary, antitumor property of emodin seems to be due to its role in regulation of redox status, inhibition of kinases, through its activation of microsomal enzymes and activation of repair enzymes in different cellular systems. But emodin’s role in interacting with protein kinases seems universal and there may exist different targets of inhibition in a given pathway. Moreover, the crosstalk among the signaling pathways makes a well-knitted chain of events ultimately bringing into control of important cellular events such as growth, differentiation, cell movement, angiogenesis, cell survival, and apoptosis. The exact final event and the magnitude of inhibition being decided by the cell type and the concentration of emodin and hence it will not be improper to prominently place emodin as a regulator of cell kinome pathway.

9. Emodin and Apoptosis—Promise to Adjunct Chemotherapy

Even though apoptosis is a common general mechanism of action elicited by emodin in tumor cells, the signaling pathways in different cells may vary. Induction of apoptosis by emodin was first reported in human renal fibroblasts probably modulated through c-myc expression. Later, a comprehensive study of apoptosis induction by emodin in lung cancer cells defined the role of cytochrome-c-mediated activation of caspase-3, bax, PKC δ and ε in emodin-induced apoptosis in CH27 and H460, promyelocytic leukemia cells. It also suggested that protein kinase C (PKC) stimulation occurs at a site downstream of caspase-3 in the emodin-mediated apoptotic pathway. In our study, emodin inhibited DNA synthesis and induced apoptosis as demonstrated by increased nuclear condensation, annexin binding, and DNA fragmentation in cervical cancer and ovarian cancer cells. Moreover, emodin-induced apoptosis was caspase-dependent and presumably through the mitochondrial pathway, as shown by the activation of caspases-3, -9, and cleavage of poly (ADP-ribose) polymerase.

Recent work on emodin-induced apoptosis has projected (i) induction of ROS by emodin leading to apoptosis and (ii) augmentation by emodin on the action of other anticancer drugs and forms an
interesting area of research in antitumor activity analysis. In various human hepatocellular carcinoma cell lines, addition of emodin led to inhibition of growth, and induction of apoptosis was mediated through ROS generation and the resultant oxidative stress. In HeLa cells, emodin enhanced arsenic induced apoptosis via generation of ROS, whereas rendered no detectable effect on normal fibroblasts. Increased ROS promoted mitochondrial transmembrane potential collapse and inhibited the activation of transcription factors NF-κB and thus inhibition of prosurvival signaling molecules. In these studies, emodin’s enhancement of drug-induced cytotoxicity was dependent on ROS generation because the enhancement of both proliferation inhibition and induction of apoptosis by co-treatment with emodin was abolished or nullified by the antioxidant N-Acetyl cysteine (NAC). The results were similar when repeated on other cells as well. Emodin could sensitize EC/CUHK1, a cell line derived from esophageal carcinoma to arsenic trioxide and the effect was comparable with a nude mouse model, with regard to their effects and mechanisms. In A549 lung carcinoma cells, emodin-mediated oxidative injury acted as an early and upstream change in the cell death cascade to antagonize cytoprotective ERK and Akt signaling, triggered mitochondrial dysfunction, Bcl-2/Bax modulation, mitochondrial cytochrome-c release, caspase activation, and subsequent apoptosis. It is also possible that the additional effect shown by co-treatment with emodin is due to the inhibition of the different tyrosine kinase activity as shown earlier. Furthermore, a recent study suggested that increased inhibition of the antiapoptotic kinase Akt activation produced by the emodin/celecoxib combination treatment plays a key role in the mechanism by which this drug combination acts to enhance cell growth suppression and apoptosis in cultured C611B ChC cells and WBneu cells. Another study on how emodin could enhance the sensitivity of tumor cells to arsenic trioxide (As2O3)-induced apoptosis using a cDNA microarray-based global transcription profiling of HeLa cells in response to As2O3/emodin co-treatment, comparing with As2O3-only treatment, found that the expression of a number of genes was substantially altered between the groups. These genes were involved in different aspects of cell function such as redox regulation, apoptosis, cell signaling, organelle functions, cell cycle progression, cytoskeleton changes, etc. These data suggest effectual cell death triggered by emodin is likely to be an outcome of interplay of all the above processes.

These promising results suggest an innovative and safe chemotherapeutic strategy that uses natural anthraquinone derivatives as ROS generators to increase the susceptibility of tumor cells to standard cytotoxic therapeutic agents. The wealth of knowledge is currently in amplification of arsenic trioxide-induced cytotoxicity in a variety of cell types. This phenomenon has earlier been shown to work efficiently for other chemotherapeutic drugs like Paclitaxel, Cisplatin, Doxorubicin, and Etoposide in HER2/neu overexpressing lung cancer and breast cancer cells. The synergistic enhancement of apoptosis, particularly when an agent is used which offers minimal toxicity to normal cells is indeed important in combination chemotherapy for cancer and will emerge to attract more attention as a promising avenue of treatment in the coming years.

10. EMODIN AS ANTIANGIOGENIC

The role of emodin in controlling angiogenesis is much less studied than its role as a growth inhibitory compound. Emodin is known to be a specific inhibitor of NF-κB, a common inflammation associated transcription factor. Most inflammatory agents activate NF-κB, which in turn results in expression of genes involved in prosurvival signaling and angiogenesis. Treatment of endothelial cells with TNF activates NF-κB; pre-incubation with emodin inhibited this activation in a dose- and time-dependent manner. Emodin’s action was thought to be an indirect effect of NF-κB and did not chemically modify NF-κB subunits but rather inhibited degradation of I-κB, an inhibitory subunit of NF-κB. It was further shown to cause G2/M arrest and induce apoptosis in endothelial cells, forming a basis of potential use of this compound in antiangiogenesis therapy. Very recent studies
probing into the mechanism of inhibition of angiogenesis by emodin reveal that emodin inhibits basic fibroblast growth factor (bFGF)-induced proliferation and migration of Human Vascular Endothelial Cells (HUVECs) and VEGF-A-induced tube formation of human dermal microvascular endothelial cells. Emodin has been shown to specifically cause protein kinase CK2 inhibition during retinal neovascularization in a mouse model of oxygen-induced retinopathy (OIR). CK2 interacts with/ and phosphorylates key signaling molecules including, p53, PTEN, p38 MAPK, PKC, phosphatidylinositol 3-kinase (PI3 kinase)-Akt pathways which are activated by angiogenic factors. Inhibition of these pathway(s) will thus lead to the amelioration of angiogenesis. Moreover, emodin is known to be an inhibitor of iNOS, which is an established initiator of angiogenesis. As days go further, a complete and clear picture of emodin’s action on angiogenesis will evolve, as of now abundant evidence is accumulating in support of emodin’s antiangiogenic effect.

11. INHIBITION OF METASTASIS BY EMODIN

Since angiogenesis is a prerequisite for proper metastasis, it is logical to expect any molecule inhibiting angiogenesis to be antimetastatic also. There are numerous reports suggesting the antimetastatic property of emodin in tumor cells. Among 44 anthraquinones tested against bacterial collagenase in vitro, emodin proved to be the most potent active inhibitor. The scientific basis of emodin’s action of inhibition of metastasis first came from the study of Zhang et al. In a study with emodin and nine derivatives, the authors identified that one methyl, one hydroxy, and one-carbonyl functional groups are critical for the biological activities of emodin.

There exists a number of reports on the beneficial effects of aloe gel for healing wounds, reducing inflammation, protecting mucous membranes, and treating ulcers. Most of these pathological conditions involve hyperactivity on the part of different matrix metalloproteinases (MMPs) and protective effect of aloe gel could be related to the presence of emodin in aloe gel and aloins. Whether it is due to a direct inhibition of these proteases or at the transcriptional level remains to be identified. Recently, aloe gel and aloins (even though emodin is not the only component present) were found as effective inhibitors of stimulated granulocyte MMPs through destabilization of MMP-8. Some reports, however, suggest upstream effect of emodin in MMP gene transcription also. Emodin inhibited the production, but not activity of MMP-9 and thus inhibited the invasion and metastasis in human ovarian carcinoma HO-8910PM cells. Further research in this area seems to agree upon this point. In glioma cells, emodin effectively inhibited hyaluronic acid (HA)-induced MMP secretion and invasion through inhibition of focal adhesion kinase (FAK), ERK1/2, and Akt/PKB activation, and partial inhibition of AP-1 and NF-κB transcriptional activities. All the above data

Figure 3. A schema of mechanism of emodin action. Various regulatory factors (EGF, VEGF, interleukins, TNF-α etc) bind on cell surface receptors and transmit signals through the various membrane receptors-associated molecules leading to the activation/alteration of several growth promoting pathways as indicated in the Figure 2. This is only a general diagram. Several smaller details have been intentionally omitted for the sake of better understanding. Since most pathways are shared between different biological processes such as proliferation, cell migration, apoptosis, survival, etc., and hence emodin can interfere with more than one pathway, the final effect will depend on the cumulative sum of all those pathways and on cell type and concentration of emodin. The overall figure could be summarized as below: Inhibition of the different components of MAPK pathways. Activation of reactive oxygen species, which leads to the formation of (i) mitochondrial stress mediated as well as (ii) SAPK/JNK pathways, etc. Inhibition of cell migratory pathways, the mediators are more or less same as in the cell growth process. Inhibition of cell survival through inhibition of NF-κB family of proteins. Regulation of cell cycle mediators leading to the stalling of cells. Activation of p38MAPK signaling leading to cell removal, if the damage is irreparable. Activation of proteosomal degradation; disruption of Androgen Receptor (AR)-HSP90 complex and release of AR to bind with MDM2 for ubiquitination and subsequent proteosomal degradation inhibiting AR-dependent growth. Inhibition of PI3K-Akt pathway, which is important in the inhibition of apoptosis, therefore inhibition of this pathway ensures easy cell removal. Blue dotted lines indicate activating pathways. Blunt ended black dotted lines show blocking pathways. Green arrows (gradient) show the endpoints of the indicated pathways (such as growth, migration, etc). Blue double-headed arrows indicate the cross talks between the pathways; these crosstalks exist at different levels and the arrows only serve as overall indicators and necessarily do not mean interaction between the partners only as shown in the figure.
Figure 3.
suggest that emodin could be a possible target for inhibition of metastasis in a variety of tumors by multiple mechanisms. To conclude, emodin at higher concentrations eliminates the cells by direct cytotoxicity and at much lower quantities, inhibits tumor-associated features such as angiogenesis and metastasis. Therefore, elucidation of critical pathways in metastasis where emodin could exert its inhibitory effects should make it a perfect fit for antitumor therapy.

12. CONCLUSION

To obtain a clear picture regarding emodin’s effects on mammalian system, 2-year genetic toxicology and carcinogenesis studies of emodin were conducted by NTP of NCI, USA.41 Thus, post 2001 era has witnessed a surge of research activities done on emodin’s antitumor action in a variety of cell systems. A schema for the plausible mechanism of action of emodin in mammalian system is given in Figure 3. Apart from these, emodin is reported to be phototoxic in vitro.128 By a phototoxic analysis, by using Chinese hamster V79 cells, emodin was found to be photochemical toxic and not photochemical genotoxic.129 A scrupulous analysis of biological properties of emodin such as genotoxic, (at least in microbes), anti-inflammatory, chemopreventive, cell cycle inhibitory, as inhibitor of different protein kinases, as an antitumor agent, inducer of apoptosis in tumor cells, inhibitor of key regulators in angiogenesis pathways and metastasis, shows its efficiency as an agent controlling various cellular processes. Recent years have thus witnessed a reappraisal of different modes of use of emodin in the elimination/retardation of growth of tumor cells either alone or in combination with other chemotherapeutic agents. This has become possible because more targets have been identified with newer pathways discovered for tumor growth or metastasis. Gene expression screening by microarray has also contributed to find alternate modes of drug action and also to understand the often-found crosstalks between pathways. The concept of synergism of cytotoxicity by a relatively non-toxic compound or with moderate toxicity with a standard drug has opened better anticancer treatment options, even though most of these are still in experimental stage. Our up-to-date summary of emodin, its activities and potentials, is aimed to contribute to this process.

Although the results from this review will be quite encouraging for the use of emodin as an adjunct antitumor agent, several limitations currently exist in the current scenario. Some of the studies were conducted using plant extracts containing emodin. In such studies, the biological effect observed may be due to the combined effects of various components. While a lot of in vitro studies have successfully been carried out showing its efficacy as antitumor agent, more trials have to be done to generate authentic data. At present, co-treatment data exists only for As2O3 and more drugs needs to be recruited for evaluating emodin’s synergistic potential, that too in different cell systems. No information on the absorption, distribution, metabolism, and excretion, or on toxicity or carcinogenicity of emodin in humans is found in a search of the available literature. There is ample information we have from animal and experimental studies. However, there may exist differences in pharmacokinetics and biological effects between man and rodents. To cite an example, emodin is not found as a cathartic in rodents possibly due to a different gut structure from humans and with certainly different reabsorption capability in colonic function.41 However, such differences will always exist and the use of newer compounds for curbing cancer will depend on the quantum of existing and available data on their toxicities and when conventional agents stall in front of a refractory/non-responsive malignancy.

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