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The Chemopreventive Effects of Natural Products Against Human Cancer Cells

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Abstract: There is accumulating scientific evidence that many of the natural herbs have medicinal properties that can alleviate symptoms or prevent disease. A growing number of researches have demonstrated that commonly used herbs such as green tea, licorice, *Cirisium rhinoceros* Nakai, cloves, *Terminalia arjuna* Linn., *Euphorbia jolkini* Bioss, *Polygonum cuspidatum*s, *Myrica rubra* Sieb et Zucc, *Centella asiatica*, *Bupleurum kaoi* , *Ochrosia elliptica* Labill, *Stephania tetrandra*, and *Rhei Rhizoma* possess chemopreventive properties that, in some cases, can be used therapeutically. These herbs have been found to possess significant antiproliferative activity against various cancer cells and this activity is supposed to be associated with the modulation of cell cycle progression and induction of apoptosis. In this review, we summarize our findings from studies performed to date regarding the chemopreventive activities of the above-mentioned herbs and their ingredients on the various types of cancer cells.

Keywords: cancer; cell cycle; apoptosis; natural product.

1. Introduction

Cancer remains a major public health problem in the world. The disease is responsible for approximately several million deaths annually, mainly in underdeveloped and developing countries [1-4]. Effective chemopreventive treatment for cancer could have an important impact on cancers cancer morbidity and mortality, such as that of breast cancer. Nowadays, chemoprevention is gaining more attention. This approach aims to decrease overall cancer morbidity and mortality by using substances that are capable of preventing cancer progression. Several classes of natural compounds have been evaluated for this purpose. Each of these classes of plant-derived compounds or extract interacts with the host to confer a preventive benefit by regulating cellular signaling of proliferation and death.

Apoptosis has been characterized as a fundamental cellular activity to maintain the physiological balance of the organism. It is also involved in immune defense machinery and plays an important role as a protective mechanism against carcinogenesis by eliminating damaged cells or abnormal excess cells proliferated owing to various chemical agents' induction [5-7]. Emerging evidence has demonstrated that the anticancer activities

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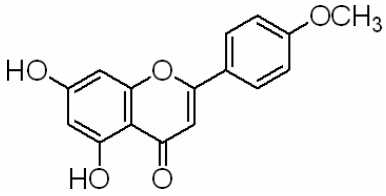
of certain chemotherapeutic agents are involved in the induction of apoptosis, which is regarded as the preferred way to manage cancer [8]. Apoptosis signaling converges at the activation of initiator caspases (i.e., caspase-8 and caspase-9), which leads to the proteolytic activation of effector caspase (i.e. caspase-3) which cleaves the cellular substrate, resulting in cell death [6, 7]. The death receptor pathway is triggered by members of the death receptor superfamily, such as Fas/APO-1. Ligation of Fas/APO-1 by agonistic antibody or its mature ligand (Fas ligand) induces receptor oligomerization and formation of death-inducing signaling complex (DISC), followed by activation of caspase-8. The mitochondrial apoptotic pathway is controlled by Bcl-2 family protein, including the proapoptotic Bax and antiapoptotic Bcl-2 and Bcl-X_L. Death stimuli induce the release of cytochrome *c*, procaspase-9, and other proapoptotic factors from the mitochondria into the cytoplasm, thereby activating downstream effector caspases such as caspase-3 [5-7]. Cross-talk between death receptor and mitochondrial pathways is provided by Bid (a member of Bcl-2 family). Caspase-8-mediated cleavage of Bid greatly increases its pro-death activity and results in its translocation to the mitochondria, where it promotes cytochrome *c* exit [9]. Tumor suppressor gene p53 is activated in response to various genotoxic stresses, resulting in cell cycle arrest or apoptosis. Functional p53 protein has shown that it is a transcription factor with sequence-specific DNA binding activity [10-11]. Cell-cycle arrest that is dependent on p53 requires transactivation of p21/WAF1, Gadd45 and cyclin G. The induction of p21/WAF1 causes subsequent arrest in the G0/G1 or G2/M phase of the cell cycle by binding of cyclin-cdk complex [11]. The mechanism of p53-induced apoptosis involves initiation of the mitochondrial apoptotic pathway, death receptor signaling, and activation of caspase cascade.

This review summarizes known and proposed mechanisms of action for various chemopreventive agents of interest by highlighting their potential and mechanisms in cancer therapy and prevention.

2. Flavonoid

A) Acacetin (5,7- Dihydroxy- 4' - methoxyflavone)

Acacetin, present in *Cirisium rhinoceros* Nakai (Compositae), is used in folklore medicine [12]. Our studies showed that acacetin inhibits the proliferation of human liver and lung cancer cells, HepG2 and A549 cells, respectively, by inducing apoptosis and blocking cell cycle progression. G0/G1 arrest was associated with an increase in p53 and its target gene, p21/WAF. In addition, Fas/APO-1/Fas ligand/caspase-8 apoptotic pathway played an important role in acacetin-induced apoptosis both in Hep G2 and A549 cells [13-14].

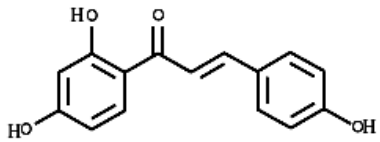


Acacetin

B) Isoliquiritigenin (4,2′,4′-Trihydroxychalcone; ISL)

Isoliquiritigenin (ISL), a flavonoid found in licorice (legume) and shallot (liliaceae), has been demonstrated to inhibit cell proliferation both in human liver cancer Hep G2 and lung cancer A549 cells [15-16]. The cell proliferation inhibition achieved by ISL treatment resulted in a G2/M-phase arrest and programmed cell death. ISL treatment was found to induce the upregulation of p53, p21/WAF1, Fas/APO-1 receptor, Fas ligand, Bax and NOXA, but this effect was not observed in Bad levels. The enhancement of p21/WAF1, Fas/APO-1, Bax and NOXA were decreased in Hep G2 cells that lack functional p53. Fur-

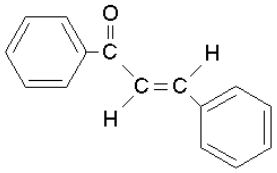
thermore, Hep G2 cells were significantly more resistant to ISL when the activity of p53 was blocked, suggesting that p53 may play a key apoptotic role, and may do so by regulating the expression of specific target molecules that promote efficient apoptotic cell death following G2/M-cell cycle arrest.



Isoliquiritigenin

C) Chalcone (1,3-Diphenyl-2-propenone)

Chalcones are precursor compounds for flavonoid synthesis in plants. Naturally occurring chalcones are mostly in the hydroxylated forms and many reports have documented their biologically active properties [17]. A recent study in our lab demonstrated that the core structure of the chalcones, chalcone (1,3-Diphenyl-2-propenone), exhibited a significant chemopreventive activity against two human breast cancer lines, MCF-7 and MDA-MB-231. Our study showed that chalcone significantly decreased the expression of cyclin B1, cyclin A and Cdc2 protein, as well as increased the expression of p21/WAF1 and p27/Kip1 in a p53-independent manner, contributing to cell cycle arrest. In addition, our results also demonstrated that chalcone initiated death receptor pathway by increasing the levels of Fas/APO-1, membrane-bound as well as soluble Fas ligand. It also triggered the mitochondrial apoptotic signaling by increasing the amount of Bax and Bak and reducing the level of Bcl-2 and Bcl-X_L, and subsequently activated caspase-9 in MCF-7 and MDA-MB-231 cells [18].



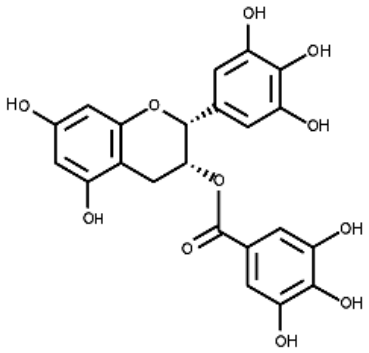
Chalcone

3. Tannins and others derivatives

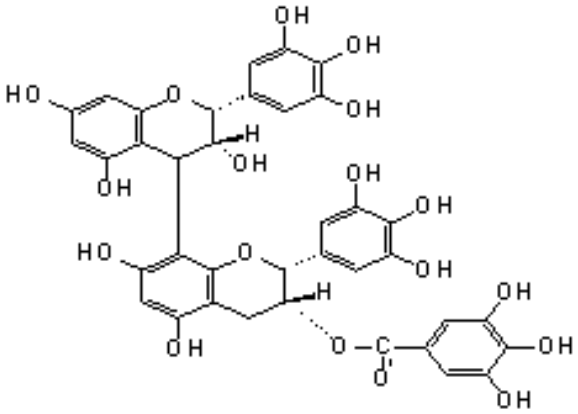
A) EGCG ((-)-epigallocatechin-3-gallate), Prodelphinidin B-2 3'-*O*-gallate

The consumption of green tea has been associated with a reduced risk of developing cancer of the ovary, oral cavity, colon, stomach, and prostate [19-21]. This beneficial health effect has been attributed to the catechins in tea: (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG) and (-)-epigallocatechin gallate (EGCG) [19]. The chemoprotective effect of EGCG has been shown to exhibit chemopreventive effects in Hep G2 cells in our studies [22]. EGCG inhibited the proliferation of Hep G2 by inducing apoptosis and blocking cell cycle progression in the G1 phase. EGCG significantly increased the expression of p21/WAF1 protein in a p53-dependent manner, and this contributed to cell cycle arrest. An enhancement in Fas/APO-1 and its two form ligands, membrane-bound Fas ligand (mFasL) and soluble Fas ligand (sFasL), as well as Bax protein, were responsible for the apoptotic effect induced by EGCG.

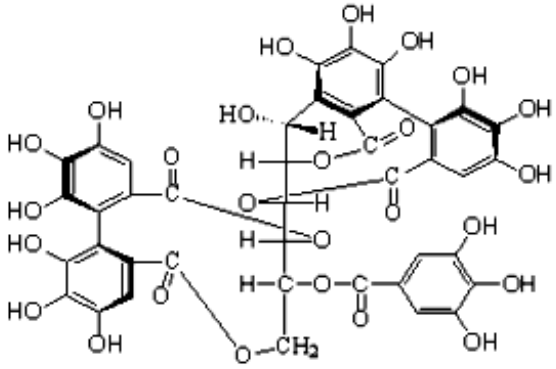
Prodelphinidin B-2 3'-*O*-gallate, a proanthocyanidin gallate also isolated from green tea leaf, has also been demonstrated for its anti-proliferative activity in human non-small cell lung cancer A549 cells by G0/G1 arrest and apoptosis induction [23].



EGCG



Prodelphinidin B-2 3'-*O*-gallate



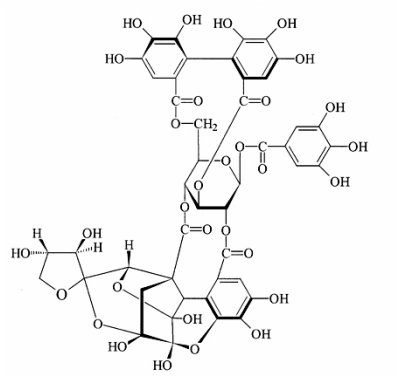
Casuarinin

B) Casuarinin

Casuarinin is isolated from the bark of *Terminalia arjuna* Linn. (Combretaceae). Our data showed that casuarinin inhibited the proliferation of human breast and lung cancer cells [24, 25]. Flow cytometric analysis indicated that casuarinin could arrest MCF-7 cells in the G0/G1 phase. The blockade of cell cycle progression was attributed to the amount of enhancement of p21/WAF1 protein in a p53-independent manner. In addition, our study also indicated that Fas ligands, mFasL and sFasL, increased in casuarinin-treated MCF-7 and A549 cells. Moreover, levels of Fas/APO-1 and the activity of caspase-8 were simultaneously enhanced in FasL-upregulating MCF-7 and A549 cells. Furthermore, when the Fas/Fas ligand system was blocked by its antagonist ZB4, a decrease in both cell growth inhibition and the pro-apoptotic effect of casuarinin was noted. Similarly, cell growth inhibition and apoptotic induction of casuarinin decreased in MCF-7 and A549 cells treated with caspase-8 inhibitor. Thus, these findings are the first to show that the Fas/FasL system plays an important role in casuarinin-mediated MCF-7 cellular apoptosis.

C) Putranjivain A

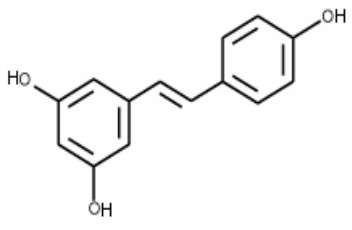
Putranjivain A, isolated from the whole plant of *Euphorbia jolkini* Bioss (Euphorbiaceae), was investigated for its antiproliferative activity in human breast adenocarcinoma MCF-7 cells [26]. The results showed that putranjivain A inhibited the proliferation of MCF-7 by blocking cell cycle progression in the G0/G1 phase and inducing apoptosis. Our study indicated that treatment of MCF-7 cells with putranjivain A had no significant changes on the expression of p53, but the amount of p21/WAF1 was increased by the same treatment. The inhibition of cyclin-cdk complex by p21/WAF1 might be amplified by simultaneous attenuation of the protein levels of cyclins (cyclin D1, D2, E) and cdks (cdk2, 4, 6), and by forming Cdk2/p21/WAF complex. Therefore, blockade of cell cycle progression by putranjivain A could be attributed to the amount of enhancement in p21/WAF1 protein expression level. In contrast, putranjivain A increased the expression of Fas, mFas ligand and sFas ligand. Fas/FasL/caspase-8 pathway involved in putranjivain A-mediated apoptosis was further supported by pretreatment of cells with the specific caspase-8 inhibitor LEHD-CHO or Fas antagonist ZB4 which inhibited the cell growth inhibition and apoptosis induction effect of putranjivain A.



Putranjivain A

D) Resveratrol (trans - 3, 4', 5-transtrihydroxystiblene)

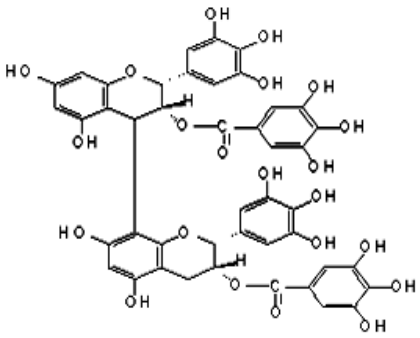
Resveratrol is one of the plant antibiotics known as phytoalexins found in grapes, nuts, fruits, red wine and *Polygonum cuspidatum*s [27]. Resveratrol is an effective antioxidant with strong anti–inflammatory and antiproliferative activity. It also shows inhibitory effects on diverse cellular processes associated with tumour initiation, promotion and progression [28]. The health benefits of resveratrol have been attributed to its antioxidant properties. The relationship between resveratrol and liver cancer risk has been reported and supported by our studies, demonstrating that the resveratrol-treated Hep G2 cells were arrested in G1 phase and underwent apoptosis [29]. In addition, we also illustrated that the resveratrol-treated cells had enhanced Bax expression but they were not involved in Fas/APO-1 apoptotic signal pathway. In contrast, when the p53-negative Hep 3B cells were treated with resveratrol, they did not show the antiproliferation effect and neither did they show significant changes in p21 nor Fas/APO-1 levels.



Resveratrol

E) Prodelphinidin B-2 3,3'-di-*O*-gallate

Myrica rubra Sieb et Zucc. (Myricaceae) is well known as a rich source of tannins. Its bark was traditionally used as an astringent, an anti-diarrhea, and also as a dyeing and tanning agent in Japan and China [30]. Prodelphinidin B-2 3,3'-di-*O*-gallate, a proanthocyanidin gallate from *Myrica rubra* was observed to arrest cell cycle at G0/G1 phase and induce apoptosis in MCF-7 and A549 cells by p21/WAF1 and Fas/APO-1/Fas ligand pathway, respectively [30, 31].



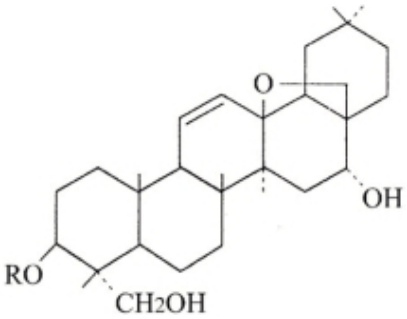
Prodelphinidin B-2 3,3'-di-*O*-gallate

4. Triterpene and saponins

A) Saikosapoin-D

Saikosaponin-D is a saponin extract derived from several species of *Bupleurum* (Umbelliferae), which is used for the treatment of various liver diseases in traditional Chinese medicine [32]. The chemoprotective effects of Saikosaponin-D against liver (Hep G2 and Hep 3B) and lung cancer (A549) cells had been demonstrated in our laboratory [33, 34]. Saikosaponin-D inhibits the cell growth of A549, Hep G2 and Hep 3B by inducing apoptosis and blocking cell cycle progression in the G0/G1 phase. Saikosaponin-D significantly increased the expression of p53 and p21/WAF1 protein, contributing to cell cycle arrest. In addition, an increase in Fas/APO-1 and its two form ligands, membrane-bound Fas ligand (mFasL) and soluble Fas ligand (sFasL), as well as Bax protein, was responsi-

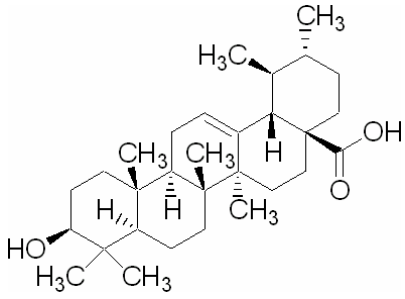
ble for the apoptotic effect induced by Saikosaponin-D. Furthermore, Saikosaponin-D also inhibited the cell survival signaling by enhancing the amount of IκBα in the cytoplasm and reducing the level and activity of NF-κB in the nucleus, and subsequently attenuated the expression of Bcl-X_L in both Hep G2 and Hep 3B cells. Saikosaponin-D therefore, decreased the cell proliferation and inducted apoptosis both in p53-postive Hep G2 and p53-negative Hep 3B cells.



Saikosaponin D

B) Ursolic acid (3β –Hydroxy-12-ursen-28-ic acid)

Ursolic acid (UA) is a pentacyclic triterpene acid widely distributed in medical herbs and edible plants, and also in wax-like coating on apples and other fruits. We have reported that UA inhibits the cell proliferation of human lung cancer cell line A549 and provided a molecular understanding of this effect [35]. The results showed that UA blocked cell cycle progression in the G1 phase that was associated with a marked decrease in the protein expression of cyclin D1, D2, and E and their activating partner cdk2, 4, and 6 with concomitant induction of p21/WAF1. This accumulation of p21/WAF1 might be through a p53-dependent manner. Further, UA treatment also resulted in the triggering of apoptosis as determined by DNA fragmentation assay. This effect was found to have correlation with the up-regulation of Fas/APO-1, Fas ligand, and Bax, and down-regulation of NF-κB, Bcl-2, and Bcl-X_L.

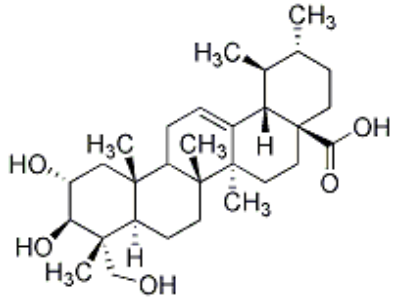


Ursolic acid

C) Asiatic acid

Asiatic acid was extracted from the tropical medicinal plant *Centella asiatica*. The acid was found to exhibit effective cell growth inhibition by inducing cancer cells to undergo S-G2/M phase arrest and apoptosis [36]. Blockade of cell cycle was associated with increased p21/WAF1 levels, and reduced amounts of cyclinB1, cyclinA, Cdc2 and Cdc25C in a p53-independent manner. Asiatic acid also reduced Cdc2 function by increasing the association of p21/WAF1/Cdc2 complex and the level of inactivated phospho-Cdc2 and phospho-Cdc25C. The acid treatment triggered the mitochondrial apoptotic pathway that was indicated by changes in Bax/Bcl-2 ratios, cytochrome *c* release and caspase-9 activation. But it did not act on Fas/APO-1/Fas ligand pathways and the activation of caspase-8. Our study also found that mitogen-activated protein kinases (MAPKs), extracellular signal-regulated kinase (ERK1/2) and p38, but not c-Jun N-terminal kinase (JNK), are critical mediators in asiatic acid-induced cell growth inhibition. U0126 or SB203580, specific inhibitors of MEK and p38 kinase activities, significantly decreased or delayed apoptosis. Asiatic acid was likely to confine the breast cancer cells in the S-G2/M phase mainly through the p38 pathway, because both SB203580 and p38 siRNA inhibition significantly attenuated the accumulation of inactive phospho-Cdc2 and phospho-Cdc25C proteins and the cell numbers of S-G2/M phase. Moreover, U0126 and ERK siRNA inhibition completely suppressed

asiatic acid-induced Bcl-2 phosphorylation and Bax upregulation, and caspase-9 activation. Taken together, these results imply a critical role of ERK1/2 and p38 but not JNK, p53, and Fas/Fas ligand in asiatic acid-induced S-G2/M arrest and apoptosis of human breast cancer cells.



Asiatic acid

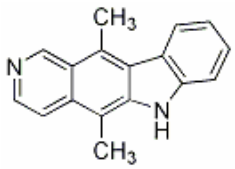
D) Saponin-enriched fraction of *Bupleurum Kaoi*

The effects of saponin-enriched fraction (SEF) from *Bupleurum Kaoi* in human non-small cell lung cancer A549 cells were investigated in our study [37]. An upregulation of Fas/APO-1 and its two forms of ligands, membrane-bound Fas ligand (mFasL) and soluble Fas ligand (sFasL), were responsible for the apoptotic effect induced by SEF.

5. Alkaloids

A) Ellipticine (5, 11 –dimethyl -6*H* -pyrido [4,3-*b*]carbazole.)

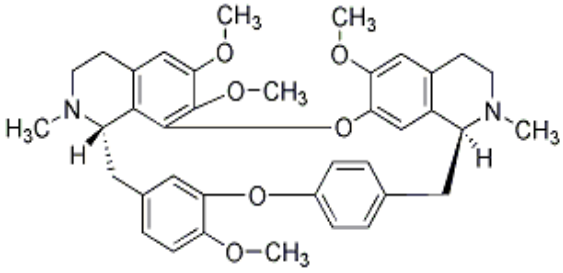
Ellipticine (5, 11 – dimethyl - 6*H* - pyrido [4,3-*b*]carbazole.) is one of the simplest naturally occurring alkaloids, having a planar structure. It was first isolated in 1959 from the leaves of the evergreen tree *Ochrosia elliptica* Labill (Apocynaceae), which grows in the wild in Oceania [38]. We have assessed that the molecular mechanisms during ellipticine-mediated growth inhibition and induction of apoptosis in human breast (MCF-7 and MDA-MB-231) and liver (Hep G2) cancer cells were due to cell cycle arrest and induction apoptosis [39-41]. This inhibition of ellipticine on cell cycle progression was associated with a marked increase in the protein expression of p53 and, p27/Kip1, but not of p21/WAF1 in MCF-7 cells. Ellipticine treatment increased the expression of Fas/APO-1 and its ligands, mFas ligand and sFas ligand, and subsequently induced activation of caspase-8. It also triggered mitochondria apoptotic pathway by regulation of the Bcl-2 family proteins expression. The mitochondrial apoptotic pathway amplified the Fas/APO-1/Fas ligand death receptor pathway by Bid interaction [39].



Ellipticine

B) Tetrandrine ((1β)-6,6',7,12-Tetramethoxy-2,2'-dimethylberbaman))

Tetrandrine, a bis-benzylisoquinoline alkaloid, is isolated from the root of *Stephania tetrandra* (S. Moore) [42]. The effect of tetrandrine in the human liver cancer line Hep G2 was investigated in our laboratory [43]. The results showed that tetrandrine not only inhibited Hep G2 growth but also induced apoptosis and blocked cell cycle progression in the G1 phase. Both p53 upregulation and Fas/APO-1/FasL apoptotic system possibly participated in the cell cycle inhibition and apoptosis induction by tetrandrine in Hep G2 cells, respectively.

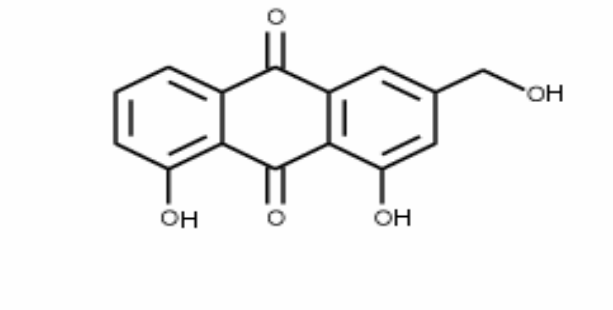


Tetrandrine

6. Anthraquinone compound

A) Aloe-emodin (1,8-dihydroxy-3-(hydroxy -methyl-9,10-anthracenedione)

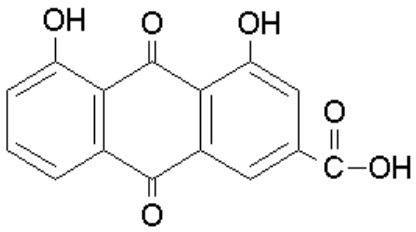
Aloe-emodin is present in some traditional medicinal plants such as *Rhei Rhizoma* [44]. Our study has investigated the anticancer effect of aloe-emodin in two human liver cancer cell lines, Hep G2 and Hep 3B [45]. We observed that aloe-emodin inhibited cell proliferation and induced apoptosis in both the cell lines examined, but with different antiproliferative mechanisms. In Hep G2 cells, aloe-emodin induced p53 expression and was accompanied by induction of p21/WAF1 expression that was associated with a cell cycle arrest in G1 phase. In addition, aloe-emodin had a marked increase in Fas/APO1 receptor and Bax expression. In contrast, with p53-deficient Hep 3B cells, the inhibition of cell proliferation of aloe-emodin was mediated through a p21-dependent manner that did not cause cell cycle arrest or an increase in the level of Fas/APO-1 receptor, but rather promoted aloe-emodin induced apoptosis by enhancing expression of Bax. These findings suggest that aloe-emodin may be useful in liver cancer prevention.



Aloe-emodin

B) Rhein (4,5-dihydroxy- anthraquinone-2 -carboxylic acid)

Rhein is an anthraquinone compound that is present in many medicinal plants. It is the major bioactive constituent of the rhizome of rhubarb (*R. palmatum* L. or *R. tanguticum* Maxim), a popular ingredient in traditional Chinese medicine for use as laxative and stomachic [46]. The effect of rhein on the human liver cancer cell line Hep G2 was demonstrated that rhein inhibited Hep G2 cell growth by inducing apoptosis and blocking cell cycle progression in the G1 phase [47]. Our data also showed that rhein significantly increased the expression of p53 and p21/WAF1 protein, which caused cell cycle arrest. An enhancement in Fas and its two forms of ligands, membrane-bound Fas ligand and soluble Fas ligand, might be responsible for the apoptotic effect induced by rhein.



Rhein

7. The extract of *Antrodia cinnamomea*

The fruiting body of *Antrodia camphorata* is well known in Taiwan as a traditional medicine for treating cancer and inflammation [48, 49]. Treatment with ethylacetate extract of *A. camphorata* (EAC) decreased the cell growth of Hep G2 and PLC/PRF/5 cells in a dose dependent manner [50]. In Fas/APO-1 positive-Hep G2 cells, EAC increased the expression level of Fas/APO-1, membrane-bound Fas ligand (mFasL) and soluble Fas ligand (sFasL) in a p53-independent manner. In addition, EAC also initiated mitochondrial apoptotic pathway through regulation of Bcl-2 family proteins expression, release of cytochrome *c*, and activation of caspase-9 both in Hep G2 and PLC/PRF/5 cells. Furthermore, EAC also inhibited the cell survival signaling by enhancing the amount of IκBα in the cytoplasm and reducing the level and activity of NF-κB in the nucleus, and subsequently attenuated the expression of

Bcl-X_L in Hep G2 and PLC/PRF/5 cells. EAC therefore decreased cell growth and induced apoptosis both in Hep G2 and PLC/PRF/5 cells.

8. Conclusion

The natural products or compounds from various natural herbs described in this review are all potential candidates for development of chemopreventive and chemotherapeutic agents against cancer. Understanding the molecular mechanism of action of these natural products and their effects on cellular signaling processes as well as their structure-activity relationships is necessary for the development of new derivatives with more favorable profile in antiproliferative and chemopreventive activities.

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