MINI-REVIEW

Roles of Plant Extracts and Constituents in Cervical Cancer Therapy

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Abstract

Cervical cancer is a major health problem worldwide and is the most frequent cause of cancer in women in India. Early detection and affordable drugs with clinical efficacy have to go hand-in-hand in order to comprehensibly address this serious health challenge. Plant-based drugs with potent anticancer effects should add to the efforts to find a cheap drug with limited clinical side effects. Keeping this very purpose in mind, an attempt has been made in this review to explore the potential of plant extracts or constituents known to exhibit antitumorigenic activity or exert cytotoxic effect in human cervical carcinoma cells. Alkaloids such as those isolated from *C. vincetoxicum* and *T. Tanakae*, naucleaorals A and B, isolated from the roots of *N. orientalis*, (6aR)-normecambroline, isolated from the bark of *N. dealbata* appear promising in different human cervical carcinoma cells with the IC_{50} of 4.0-8 μg/mL. However, other compounds such as rhinacanthone and neolignans isolated from different plants are not far behind and kill cervical cancer cells at a very low concentrations. Among plant extracts or its constituents that enhance the effect of known anticancer drugs, noni, derived from the plant *M. citrifolia* perhaps is the best candidate. The cytotoxic potency and apoptotic index of cisplatin was found to significantly enhanced in combination with noni in different human cervical carcinoma cells and it therefore holds significance as promising herbal-based anticancer agent. However, efficacy needs to be further investigated in various cervical cell lines and more importantly, in *in vivo* cervical cancer models for possible use as an alternative and safe anticancer drug

Keywords: Cervical carcinoma cells - plant extracts / constituents - alkaloids - cytotoxicity.

Asian Pacific J Cancer Prev, **14 (6)**, 3429-3436

Introduction

Cervical cancers, like any other cancers, continue to be pose serious health problem globally. The scenario is worst in developing countries or wealthy country with low income groups. Improvement in cervical cancer screening and treatment for adult women and to successfully vaccinate girls against human papillomavirus (HPV), the virus that causes cervical cancer is the priority. Since the cost issue of the vaccine is a concern, it is imperative that all possible resources are explored to treat the disease. Plants and their constituent are being tested for their clinically efficacy for the treatment of cervical cancer which have to go hand-in-hand with the screening program to detect early lesions of the cancer. Keeping this very essence in mind, attempt has been made in this review to explore the potential of plant extracts or its constituents known to exhibit antitumorigenic activity or exert cytotoxic effect in human cervical carcinoma cells. Identification of potentially effective plant-based product as a potent candidate (s) against cervical cancer cells will go long way for possible therapeutic usage.

The onset for the hunt of effective alternative or natural drugs for cervical cancers was largely due to the fact that prognosis of synthetic drugs was very poor. This was aided by the high cost of these drugs making it difficult for the poor populace to get access to it. Among the few plant-based drugs tested in the beginning of this quest were diterpenoid plant products such as topotecan. It was reported to be a topoisomerase inhibitors and have been reported to be promising antitumor agent for cervical cancer (Yakushiji et al., 1997). Since then several plant extracts or its constituent have been investigated against cervical cancer cells.

Examples

Alkaloids

Seven alkaloids have been isolated from *Cynanchum vincetoxicum* and *Tylophora tanakae*, showing similar cytotoxic property against human cervical carcinoma cells, KB3-1 (Staerk et al., 2002). The IC_{50} (the concentration which caused a half maximal inhibition of cell proliferation) value for these compounds was between 7-17 nM. It was shown in this study that a rigid phenanthrene structure present in some of these alkaloids is a prerequisite for

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a high cytotoxicity of the free bases, reiterating earlier findings for the *N*-oxide alkaloids (Staerk et al., 2000). Additionally, it was also shown in the same study that presence of a free phenolic function at C-6 or C-7 show significantly high cytotoxicity toward the KB cancer cell line as in the case of some of the alkaloids. In this same cell line, another alkaloid-crebanine, isolated from the tuber of *Stephania venosa*, has also been investigated for its anticancer properties *in vitro* (Wongsirisin et al., 2012). Here, cells treated with 70.7 μM of crebanine for 48 h demonstrated inhibition in cell growth, with the IC_{50} of 24 μg/mL. The inhibition of cell proliferation and viability was shown to be through cell cycle arrest in G0/G1 phase and eventual apoptosis. Apoptotic induction by crebanine was reported to occur through caspases activation. Two isomeric indole alkaloids, naucleaorals A and B, isolated from the roots of *Nauclea orientalis* demonstrated cytotoxicity in another human cervical carcinoma cell line-HeLa (Sichaem et al., 2010). In this study, both the compounds showed significant cytotoxicity to HeLa cells with an IC_{50} value of 4.0 and 7.8 μg/mL, respectively. Another alkaloid, (6aR)-normecambroline, isolated from the bark of *Neolitsea dealbata* has also been shown to inhibit HeLa cells with an IC_{50} of 4.0 μM. (Tran et al., 2010).

Flavonoids

Apigenin, a widely distributed plant flavonoid has also been shown to inhibit the growth of HeLa cells and was reported to be a potential antitumor agent (Zheng et al., 2005). Significant reduction in the viability of HeLa cells by apigenin at 37-74 μM was observed and the IC_{50} value was found to be 35.89 μM. It was also reported in this study that apigenin acted by triggering the apoptotic pathway, characterized by induction of G_1 arrest, DNA fragmentation, increased expression of p21/WAF1, caspase-3, and some other mediators of apoptosis and also decreased in the protein expression of anti-apoptotic factor-the Bcl-2 protein. Another flavonoid with potential anticancer activity is silymarin. It is the active component of milk thistle (*Silybum marianum*), consisting of a mixture of flavanoid complexes and have been found to exert anticarcinogenic effects in preclinical trials. It was reported that the anticancer property was indicated by its ability to inhibit the growth of cancer cells including cervical cancer cells (Post-White et al., 2007).

Polyphenols

The polyphenol-rich fractions obtained from the extracts of rowan berries, raspberry, lingonberry, cloudberry, arctic bramble, and strawberry were found to be potent inhibitor of the proliferation of HeLa cells (McDougall et al., 2008). The effectiveness was found to be in the order of strawberry>arctic bramble>lingonberry>cloudberry. The IC_{50} value for strawberry, arctic bramble, lingonberry and cloudberry was found to be 25.5, 26.4, 28.7 and 31.6 μg/mL, respectively. However, rowan berries and raspberry were also able to reducing viability to ≤50% of control at 50 μg/mL. It was reported in this investigation that the all these fruit extracts except lingonberry, exhibited antiproliferative activity due to their high content of ellagitannins. Ellagitannins and ellagitannin-rich fruit extracts have also been reported earlier to inhibit cancer cell growth in other studies, possibly due to release of the potent antiproliferative compound, ellagic acid under physiological conditions (Castonguay et al., 1997; Losso et al., 2004; Larrosa et al., 2006; Misikangas et al., 2007; Ross et al., 2007; Seeram et al., 2007). On the other hand, the tannin-rich extract of lingonberry, which consisted almost entirely of proanthocyanidins, was reported to be the constituents largely responsible for the antiproliferative effects (McDougall et al., 2008). Other polyphenolic compound such as ethyl gallate, isolated from ethanol extract of *Acacia nilotica* Wild. Ex. Del. leaves showed cytotoxic effect on HeLa cell lines as revealed by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cell proliferation assay. It was found that the compound was moderately active and IC_{50} value was found to be 72 μg/mL for HeLa cells (1×10^5 cells/well) (Kalaivani et al., 2011). In another study involving ellagic acid, it was reported that HeLa cells exposed to 3 Gy of gamma radiation result in ROS-mediated cell toxicity and decrease in cell viability as indicated by MTT assay (Girdhani et al., 2005). It was proposed in this study that the cytotoxic effect of ellagic acid was is through up-regulation of oxidative stress. Compounds such as 6-Methoxygossypol and 6, 6'-dimethoxygossypol, isolated from the root tissue of cotton plant, also belongs to the polyphenolic group, and have been shown to exhibit dose-dependent growth inhibition against another cervical cancer cell line-SiHa (Wang et al., 2008). The IC_{50} value was reported to be around 10 ppm for both the compounds.

Naphthoquinone esters

Rhinacanthins-C, -N and -Q isolated from dried roots of *Rhinacanthus nasutus*, a plant traditionally used in Thai folk medicine for treating various cancers including cervical and hepatocellular cancers, has been shown to suppressed HeLa cells through induction of apoptosis by activation of caspase-3 pathway (Siripong et al., 2006a). The same group have also shown that when these naphthoquinone esters are liposomalized with egg yolk phosphatidylcholine (EPC) and egg yolk phosphatidylglycerol (EPG), there was significant increase in the antiproliferative effects on HeLa cells as measured by cell proliferation assay (Siripong et al., 2006b). The proliferation of the cells was significantly inhibited by rhinacanthins when the concentration of these drugs was increased from 3-300 mM and the exposure time was prolonged to 24-72 h. The IC_{50} values of rhinacanthins-C, -N and -Q were 32, 17 and 70 mM for 24 h; 19, 17 and 52 mM for 48 h; and 2.7, 2.0 and 5.0 mM for 72 h incubation, respectively. In this study it was observed that liposomal rhinacanthins damaged cells in a time-dependent manner, the highest being after the 72 h incubation. It was also reported in the same study that rhinacanthins-C, -N and -Q suppressed the cell growth by cycle arrest during the G2/M phase that helps to prevent further damage and give the cell time to repair the defect, or undergoes apoptosis.

In another work involving rhinacanthone (3,4-dihydro-3,3-dimethyl-2*H*-naphthol-[1,2-*b*]pyran-5,6-dione), one

of the main bioactive naphthoquinones, isolated from *Rhinacanthus nasutus* possess cytotoxic activity in HeLa cells as indicated by MTT assay (Siripong et al., 2009). Treatment of cell culture with rhinacanthone resulted in a significant decrease in cell proliferation in a dose-dependent manner. The IC_{50} values of rhinacanthone were 5.5±0.86, 4.5±0.38, 2.5±0.37, 2.0±0.72, 2.0±0.36 and 1.2±0.1 mM for 2, 4, 8, 12, 24 and 48 h of incubation, respectively. It was also revealed by morphological changes, DNA fragmentation, cell cycle progression, TUNEL (Terminal deoxynucleotidyl transferase-mediated d-UTP Nick End Labeling) staining assay and other assays that rhinacanthone induced apoptosis in HeLa cells. It was therefore, reported that rhinacanthone induced apoptotic cell death through multiple pathways such as cell cycle arrest in G2/M phase, modulation Bcl-2 family, down-regulation of surviving and up-regulation of apoptosis inducing factor (AIF protein) as well as activations of mitochondria mediated caspase-dependent and caspase-independent signalling pathways. Azaanthraquinone compound such as laoticuzanone A, isolated from the stems of *Goniothalamus laoticus* have also been shown to exhibit cytotoxicity against HeLa cells with IC_{50} values of 0.50 µg/ml (Tip-Pyang et al., 2010). Diospyrin, a bisnaphthoquinonoid natural product show similar result of apotosis induction, chromatin condensation and nuclear fragmentation on HeLa cells and the apoptosis was believed to be mediated via activation of caspase-3 and caspase-8 (Chakrabarty et al., 2002).

Phorbol esters

Phorbol esters (PEs) isolated from the meal prepared from the kernel of *Jatropha curcas* has also been studied for its cytotoxic effects and mode of actions in HeLa cell line (Oskoueian et al., 2012). There was a dose-dependent reduction in cells proliferation by the isolated PEs. It was found in this case that the IC_{50} of 133.0±1.96 μg phorbol 12-myristate 13-acetate (PMA) equivalents/mL, while the values for the PMA as positive control were 119.6±3.73 μg/mL. Significant morphological changes were observed microscopically that resemble apoptosis in the cell line when treated with PEs and PMA at IC_{50} concentration after 24 h which were confirmed by flow cytometry analysis and DNA fragmentation. It was also reported in this study that the PEs isolated from *Jatropha* meal activated the phosphokinase-C delta (PKC-δ) and down-regulated the proto-oncogenes (c-Myc, c-Fos and c-Jun), suggesting that these changes probably led to the activation of caspase-3 protein, and apoptosis occurred in the HeLa cell lines upon 24 h treatment with PEs and PMA. It was suggested that probably phorbol esters of *Jatropha* meal could be promising as an alternative to replace the chemotherapeutic drugs for cancer therapy.

Examples of different extracts of plants or other compounds derived from it that exhibit cytotoxic properties to cervical cancer cells

Aqueous extracts

The aqueous extracts of cactus pear, the fruit of Arizona cactus (*Opuntia ficus-indica*) has been reported to exhibit cytotoxic effect in HeLa cells (Zou et al., 2005). It was shown in this study that 1% cactus pear solution inhibited 40-60% of immortalized HeLa cells, and 5% of the solution kills almost 100% of the cells at day 5. The IC_{50} after 5-day treatment with cactus pear solution was 1.8%. It was also shown that cactus pear solution induced apoptosis in HeLa, tested by TUNEL analysis. The apoptosis cell population increased by more than 50% at the concentration of 25% cactus extract compared with the untreated cells. It was also reported here that the cactus pear extracts also affected cell cycle in HeLa cells starting at a 5% concentration. The cactus extracts increased cells in G1 and decreased those in the S phase.

Methanolic extracts

Extracts prepared from leaves, stems and branches of *Atriplex confertifolia* have been shown to kill HeLa cell line, but do not affect monocyte control cells (Capua et al., 2010). It was reported in this investigation that the majority of toxic compounds were found in the polar methanol/water fractions of this plant extract which resulted into 90% death of the cells. The cell cultures incubated for 24 h with the extract display the onset of mortality at approximately 8-10 hours after incubation in this case.

In a remarkable study conducted by Booth et al. (2012) cytotoxicity of methanolic extracts of 55 species of plants-medicinal and non-medicinal were tested against HeLa cells. Out of 46 medicinal plant extracts, it was found that 80% of the medicinal plant extracts showed some type of cytotoxicity (54% were active, 26% were mildly active) suggesting some connection between plants known from indigenous cultures to have medicinal properties compared to empirically determined cytotoxicity. The nine non-medicinal plants also tended to be bioactive, with 50% active, 13% mild and 37% not active. At the same time, it was also reported that the plant families of Asteraceae, Labiatae, Pinaceae, and Chenopodiaceae were particularly active against human cervical cancer cells.

Ethanolic extracts

Neolignans isolated from ethanolic extracts of the aerial parts of *Saururus chinensis* has shown anti-proliferative property in another human cervical cancer cell line-C33a (Lee et al., 2012). The IC_{50} was found to be within 0.01 μM-2.80 μM as indicated by cell proliferation assay without any remarkable cytotoxic effects on human normal lung cells as a control.

Ethanolic extracts of medicinal plants of Thailand such as *Coscinium fenestratum* (stems) and *Kalanchoe pinnata* (leaves) has also been found to possess *in vitro* cytotoxic activity in KB3-1, derived from human papilloma virus infected cells (Kaewpiboon et al., 2012). Both extracts exhibited high cytotoxicity (IC_{50} values of ~2.18 µg/mL) and inhibited the growth of this cell line. It was proposed in this study that the ethanol extracts affect the regulation of some viral proteins that control cell division. Since the crude ethanol extracts was quite active, it was suggested that the bioactive components might have moderate to low polarity.

Dichloromethane extracts

Goniothalamin (GTN), a natural occurring styryl-lactone and dichloromethane (DCM) extract from root of *Goniothalamus macrophyllus* have been shown to be cytotoxic against HeLa cells but not against normal mouse fibroblast cells used as control (Alabsi et al., 2012). Using MTT cell viability assay, it was shown that the IC_{50} values of gonoithalamin concentrations was 3.2±0.72 µl/ml compared to untreated cells. The cell proliferation assay also indicated significant reduction in viable cell number in cultures treated with GTN at IC_{50} concentrations as compared to the controls in day 1-day 3. It was proposed in this study that GTN exhibit anti-cancer property via induction of apoptosis and causes cell cycle arrest and cell death at S phase.

Biologically active secondary metabolites from the DCM extract of stem bark of *Mesua beccariana* such as stigmasterol has been reported to possess effective cytotoxicity in preliminary tests *in vitro* against HeLa cells. Stigmasterol exhibited strong inhibition of cell proliferation, an indication of its effectiveness as anti-cancer lead compounds (Teh et al., 2012).

Some other medicinal plants of Thailand such as *Bauhinia strychnifolia* (vines) and previously mentioned *C. fenestratum* (stems) were also found to possess *in vitro* cytotoxic activity in KB3-1 (Kaewpiboon et al., 2012). The highest cytotoxic activity for vines of *B. Strychnifolia* (IC_{50} value of 1.86 µg/ml) was obtained in the DCM extract. For *C. fenestratum*, the crude DCM extracts showed high cytotoxicity (IC_{50} values of 3.25 µg/mL). The high cytotoxic activity in crude DCM extracts corroborated the earlier observation in the same study on other plant that the bioactive components have a moderate to low polarity.

Hexane extracts

Other biologically active secondary metabolites from the hexane extract of stem bark of *Mesua beccariana* such as beccamarin has been reported to possess effective cytotoxicity in preliminary tests *in vitro* against HeLa cells. Even this compound exhibited strong inhibition of cell proliferation, indicating its effectiveness as another anti-cancer lead compounds from the same plant (Teh et al., 2012). In another study, the crude hexane extract of the vines of *B. Strychnifolia* have also been found to be highly cytotoxic (IC_{50} value of 1.86 µg/ml) in KB3-1 (Kaewpiboon et al., 2012).

Chloroform extracts

Isoatriplicolide tiglate (PCAC), isolated from the chloroform soluble fraction of the leaves of *Paulownia coreana* exhibited antiproliferation activity in cervical cancer cell lines (Jung et al., 2012). The *in vitro* experiments showed that PCAC suppresses the cell growth and proliferation of cancer cells at a relatively low concentration (<10 μg/mL) and induces apoptosis at a high concentration (>50 μg/mL). It also revealed that concentration higher than 50 μg/mL induces an increase in the percentage of apoptotic cells in a time dependent manner. PCAC treatment activated caspase-8, -9, and -3, the main regulators of apoptotic cell death. It was concluded that PCAC can act as an antiproliferation agents particularly against cervical cancers by inducing cell cycle arrest in the S/G2 phase and caspase-dependent apoptosis.

Acetone extracts

Acetone extracts of *Origanum vulgare* (Oregano) and *Laurus nobilis* (Bay leaf) have also been tested for their *in vitro* cytotoxicity against HeLa cell line (Berrington & Lall, 2012). Cytotoxicity was measured using the sodium 2,3,-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)-carbonyl]-2H-tetrazolium (XTT) colorimetric assay and was found that both these plant extracts strongly inhibited the proliferation of the HeLa cells. H & E staining and confocal analysis showed the appearance of typical morphological features of apoptosis such as hypercondensed chromatin and the degradation of DNA.

Yuk-Hap Tang (Angellicae gigantis; Cnidii rhizoma; Paeoniae lactiflorae; Rehmanniae rhizoma)

YHT has been found to be effective in killing of HeLa in cytotoxic study as assessed by MTT assay (Chae et al., 2004). It was also found that YHT activated caspase-3, -6 and -9 very significantly. It also increased the pro-apoptotic protein, Bax and the anti-apoptotic protein, Bcl-2. It was further reported that YHT decreased the expression of Mn-SOD protein and its activity in HeLa cells. It was also demonstrated that YHT induces the apoptosis of HeLa cells by intervening Mn-SOD.

Six 1-glyceryl ethers

Ceratodictyols A and B and mixtures of ceratodictyols C and D and ceratodictyols E and F, isolated from the red alga-sponge assemblage *Ceratodictyon spongiosum/ Haliclona cymaeformis* have also been shown to contain cytotoxic activity against HeLa cells with an IC_{50} value of 67 µM for each (Akiyama et al., 2009).

RCE-4

RCE-4 isolated from *Reineckia carnea* has been found to exhibit potent cytotoxicity on yet another cervical cancer cells CaSki (Wang et al., 2013). In this investigation, the IC_{50} was found to be 3.37 μM. It was also reported here that RCE-4 possesses apoptosis-inducing effect in a dose-dependent manner. Transmission electron microscopy revealed that RCE-4 treatment induced nuclear shrinkage, condensation and fragmentation. It was also reported to trigger a rapid decrease of the mitochondrial membrane potential and caused the release of cytochrome c from the mitochondria into the cytoplasm. It was suggested by the apoptotic study that RCE-4 induces mitochondrial-mediated apoptosis in CaSki cells and has the potential to be developed as an anticancer agent.

Noni, fruit juice derived from the plant Morinda citrifolia

All have been found to be potent cytotoxic and apoptosis agent in HeLa and SiHa cell lines (Gupta et al., 2013). It was reported in this study that the potency of cisplatin (CP, 10 µg/ml), the commercially available anticancer drug in combination with Noni (N, 10%, v/v) (N+CP) was enhanced significantly in these two cancer cells after 24 h of incubation. The MTT cytotoxic assay and

flow cytometry analysis indicated more than 50% decrease in survival and induction of apoptosis in comparison with control in N+CP treated cells, significantly higher than N or CP alone. Even the expression of pro-apoptotic (Bax) protein was increased maximally (66-82% in both cells) and reduction in anti-apoptotic (Bcl-2; 41-51% and Bcl-XL 48-63% in both cells) proteins in N+CP group as revealed by Western blot immunoprobing. Additionally, it was also reported that the level of p53 expression went up by 43-54% in both the cells in N+CP, which was statistically significant when compared with the controls. Moreover, the expression level of survivin, an inhibitor of apoptosis went down by 20-32% in the N+CP treated both cervical carcinoma cell lines. It was also shown in this study that the activities of both caspase-3 and -9 increase significantly in both the cell lines in N+CP compared to N or CP alone treatment.

Mechanisms

Cervical cancer is a major health problem worldwide and is the most frequent cause of cancer in women in India (Jemal et al., 2011; Pathak et al., 2012; Gupta et al., 2013). Early detection and an affordable drug with clinical efficacies have to go hand-in-hand in order to comprehensibly address this serious health challenge. Plant-based drugs with potent anticancer effect with add to the efforts to find a cheap drug with least clinical side effects. Among the pioneer plant-based drug, the alkaloid taxol is perhaps the oldest known anticancer drugs including against cervical cancer cells (Liebmann et al., 1993). With increasing exploration, other alkaloids have been discovered and isolated such as (-)-(R)-13aR-antofine, (-)-(R)-13aR-6-O-desmethylantofine, (-)-(R)-13aR-secoantofine, (-)-(R)-13aR-6-O-desmethylsecoantofine, (-)-(R)-13aalpha-tylophorine, (-)-(R)-13aalpha-7-O-desmethyltylophorine and (+)-(S)-13abeta-isotylocrebrine from *Cynanchum vincetoxicum* (first four) and *Tylophora tanakae* (last three) (Staerk et al., 2002). Because of their IC_{50} values between 7-17 nM, some of them (1st, 2nd and 6th) were considered better than that of front-line antineoplastic drugs, taxol.

Among the studies reviewed here, there are few which have indicated antitumor activity *in vivo* such as topotecan (Yakushiji et al., 1997), mixture of flavonoids complexes in milk thistle (Post-White et al., 2007) and ellagic acid (Misikangas et al., 2007; Seeram et al., 2007). Raddeanin A, a triterpenoid saponin from *Anemone raddeana* has also demonstrated good antitumor activity. Its antitumor effects was carried out by testing the inhibitory effects of raddeanin A injection on the growth of cervical carcinoma U14 cell xenografts in mice (Wang et al. 2008). It was found that when injected with raddeanin A, at a dose of 4.5 mg/kg, the growth inhibition rates of U14 cell xenografts was 61.8%. This study concluded that Raddeanin A has good antitumor activity both *in vitro* and *in vivo*, and would be a potential antitumor drug.

Most of the plant extracts or its derivatives have been shown to be effective in human cervical cancer cell lines such as HeLa as mentioned previously. The other cervical cancer cell lines where plant extracts or its derivatives have been found effective are KB3-1(Staerk et al., 2002; Kaewpiboon et al., 2012), SiHa (Wang et al., 2008; Gupta eta al., 2013), C33a (Lee et al., 2012) and CaSki (Wang et al., 2013).

Some of the plant products such as topotecan, a topoisomerase inhibitors, have been shown to be more effective than antineoplastic drugs-taxol for cervical and other cancers as well in clinical trials (Yakushiji et al., 1997). Others such as the extracts of *A. confertifolia*, has indicated the significant toxic effects in cervical cancer cells in a concentration comparable to those of the FDA-approved anti-cancer drug drug Onxol® especially at the highest doses (Capua et al., 2010). Rhinacanthone,

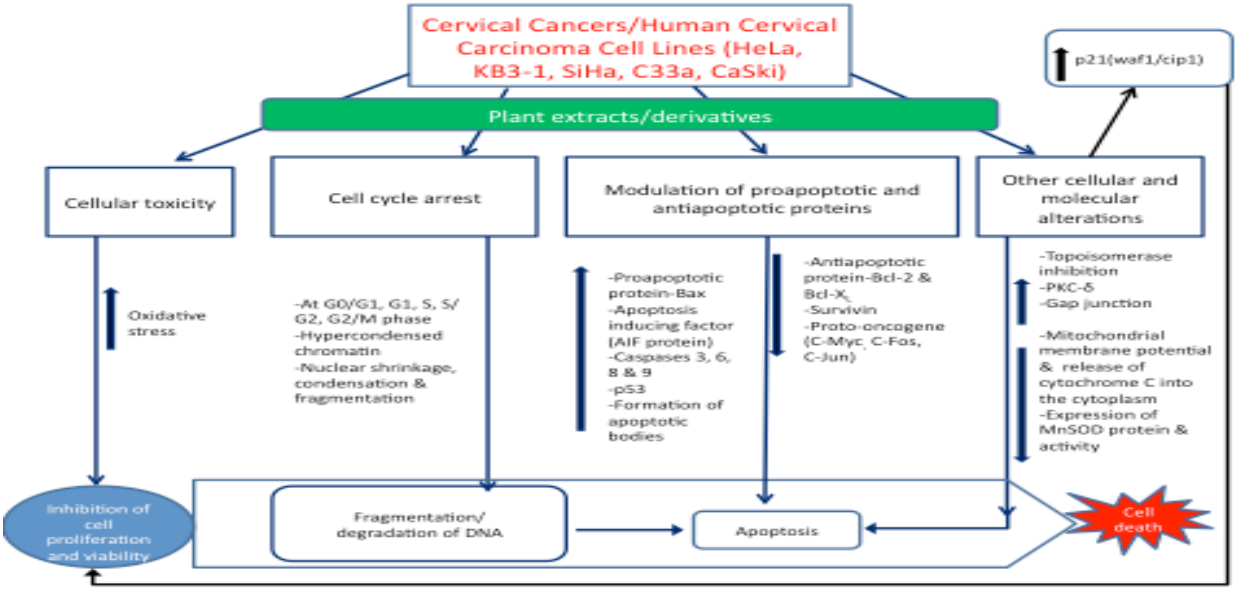

Figure 1. General Mechanism of Action of Plant Products or its Constituent on Cervical Cancer/Cervical Carcinoma Cell Lines. Bold up arrow indicate up-regulation and bold down arrow indicate down-regulation of cellular and molecular events by the plant extracts or its derivatives ultimately leading to inhibition of cell proliferation and death

bioactive compound isolated from *R. nasutus,* has been shown to possess greater efficacy than β-lapachone, an anticancer drug under similar conditions (Siripong et al., 2009).

Some of the plant constituents have been shown to enhance the toxicity of the anticancer drugs when administered together. E.g. Panax notoginseng saponins (PNS), isolated from herb notoginseng, have been reported to enhance the cancer chemotherapeutic agent-cisplatin induced cellular toxicity in transfected HeLa cells (Yu et al., 2012). In this experiment, cells seeded at high or low cell density were treated with PNS (50-200 μg/mL) for 3 h, followed by exposure to 0.5 μg/mL cisplatin plus PNS for 1 h. The clonogenic survival, assessed 7 days after exposure to cisplatin and PNS indicated that 50-200 μg/mL of PNS was non-toxic for HeLa cells while they increase the cytotoxicity of cisplatin. At 200 μg/mL of PNS, the cytotoxicity of cisplatin was enhanced by about 22%. This suggested that in combination with PNS, the dose of cisplatin could be reduced without affecting the tumoricidal efficiency leading to reduction in side effects. Other saponins such as those isolated from *Passiflora alata* and *Quillaja saponaria* exhibited strong anti-*Trichomonas vaginalis* activity (Rocha et al., 2012). It is noteworthy that *T. vaginalis*, a flagellated protozoan that causes trichomonosis, is knowm to be associated with serious health consequences including pelvic inflammatory disease, predisposition to cervical cancer, etc (Petrin et al., 1998).

It was also reported that the potency of cisplatin in combination with Noni was enhanced significantly in the cervical cancer cell lines after 24 h of incubation (Gupta et al., 2013). These finding were very significant since it was shown previously that Noni was able to inhibit the growth of tumor cells in experimental model systems (Hiramatsu et al., 1993; Liu et al., 2001; Taskin et al., 2009).

It has also been shown that BnRCH, the protein product from a novel gene isolated from *Brassica napus*, has the E3 ubiquitin-protein ligase activity, hallmark of ubiquitin-proteasome pathway (UPP) (Wan et al., 2011). In this investigation it was found that Hela cells, transiently and stably transfected with BnRCH, resulted in cell growth inhibition and increased sensitivity to the anti-cancer drug, cisplatin.

In general, the plant extracts or its constituent, cause cellular toxicity in cervical cancer cells and result in cell death by two primary pathways-cell cycle arrest and apoptosis. The arrest primarily occurs at G1 and G2/M phase although arrest at other stages has also been reported as discussed earlier. Cell cycle arrest results into fragmentation or degradation of DNA, a hallmark of induction of apoptosis. In most of the cases, it has also been reported that there is activation of caspases, particularly caspase-8, -9 and -3 leading to apoptosis. Additionally the plant extracts or its derivatives also induces the proapoptotic proteins such as Bax and represses antiapoptotic protein Bcl-2 and Bcl-XL. The other molecular modulations by plant extracts or its constituent have been summarized in Fig.1. Apart from inhibition of growth in cervical cancer cells by cell cycle arrest and induction of apoptosis, it has also been reported that other mechanism could be involved which might operate through cell cycle arrest and up-regulation of p21 (waf1/cip1) rather than apoptosis. E.g. BnRCH can modulate the UPP, an important protein degradation system universally existing in all eukaryotic organisms, causing growth inhibition by cell cycle arrest at G2 phase with the transcriptional up-causing growth inhibition by cell cycle arrest at G2 phase with the transcriptional up-regulation of p21 (waf1/cip1), without triggering apoptotic pathway (Wan et al., 2011).

Conclusions

Plant extracts or its constituents which are known to inhibit the cervical cancer cells without affecting the normal cell certainly has the potential to be used in cancer therapy. Among them that have been reviewed here, alkaloids such as those isolated from *C. vincetoxicum* and *T. Tanakae* (Staerk et al., 2002), naucleaorals A and B, isolated from the roots of *N. orientalis* (Sichaem et al., 2010), (6aR)-normecambroline, isolated from the bark of *N. dealbata* (Tran et al., 2010), have shown potent cellular toxicity in different human cervical carcinoma cells with the IC_{50} of 4.0 -8 μg/mL. This is not surprising since another alkaloid-taxol, derived from plant have been routinely used as an anticancer drug under different brand names worldwide. However, another compound-rhinacanthone isolated from *R. nasutus* has also exhibited cytotoxicity to cervical cancer cells at a very low concentration (Siripong et al., 2009). Rhinacanthone role becomes quite significant since it is known to induce apoptotic cell death through multiple pathways as mentioned previously. Recent studies points to other molecules such as neolignans isolated from ethanolic extracts of the aerial parts of *S. chinensis* which has shown very strong anti-proliferative property as mentioned earlier (Lee et al., 2012). The IC_{50} was found to be within 0.01-2.80 μM without any remarkable cytotoxic effects on human normal lung cells as a control. However, its potency against different types of cervical cancer cells needs to be tested for potential therapeutic use.

Among plant extract or its constituents that enhance the effect of known anticancer drugs, noni, derived from the plant *M. citrifolia* perhaps, is the best candidate. The cytotoxic potency of cisplatin (10 μg/ml) was found to significantly enhance in combination with noni (10%, v/v) in two human cervical carcinoma cells as discussed above (Gupta et al., 2013). The apoptotic index was also remarkably increased in combination with each other and therefore holds significance as promising herbal-based anticancer agent. It is more so since noni has been known to possess antiproliferation ability against other tumor cells in experimental model systems as reported earlier.

Notwithstanding the significance of some of these plant extracts or its constituents on the ability to kill human cervical carcinoma cells *in vitro*, the efficacies needed to be further investigated in various cervical cell lines and more importantly, in experimental animal systems. The data on animal models of cervical cancers are not too many owing to the complexity of cancers in experimental animals in general. However, more research investigations

in vivo will probably generate better understanding on the role of the plant extracts or its constituents with an aim at their possible use as an alternative and safe anticancer drug. It will even be desirable if plant-based drug by itself is not so effective but in combination with known commercially used anticancer drug, is able to enhance the effectiveness of the drug. This can result into reduction in the drug dose and the resultant side effects can be minimized.

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