Comprehensive study of molecular mechanism in Cervical Cancer cell death in vitro and in vivo induced by Phytochemicals

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Abstract

Biologically active compounds are very effective for any cancer treatment. Conventional chemotherapy has many adverse effects on human health. For this reason, researchers and Scientists are engaged for investigating new weapons for cancer treatment. Cervical cancer is the deadliest type of cancer and the second most common cancer in many countries. Treatment of cervical cancer is based on radiotherapy, chemotherapy, or combined mode of chemo and radiotherapy as well as surgery. Cytotoxicity and cell death induced by many phytochemicals have been studied extensively. Naturally occurring phytochemicals and their derivatives have special attention for the treatment of cervical cancer. The mortality rate of the disease is high among females in many countries. All chemotherapeutics more than 50% are obtained from various sources of plants. Some important phytochemicals like terpenoid, triterpenoid aloe Emodin, and curcumin have important medicinal value. In this context, bioactive compounds targeting for treatment of cancer are highly acceptable. They induce cell malignant cell death in different modes. All deaths are controlled by various signaling pathways. Such as apoptotic death, autophagy death, necrotic, and ferroptosis like this. Current advancements in phytochemical research can be able to identify the action of phytochemicals on malignant cells and the mode of cell death. It has been established that the involvement of death factors is directly or indirectly correlated with the discovery of new drugs. The bioactivity as well as the anticancer activity of phytochemicals has been extensively studied by many groups. The main objective of this review article is to accumulate molecular mechanisms of cell death by different phytochemicals in the case of cervical cancer cells in vivo and in vitro. The targeted molecular pathway of bioactive phytochemicals in cervical cancer will explore the new trail for promising and effective treatment of cervical cancer in the future. All data are collected after extensive Google Scholar, PubMed, Sci-Hub, and NIH searches.

1. Introduction

Several retroviruses have been identified in past decades, which are actively involved in malignancy in human body cells. These viruses known as onco viruses contains, which contains oncogenes and are responsible for the transformation of normal cells into malignant cells [1]. Oncogenic viruses can be categorized into different classes depending on their mode of infection and malignant cell transformation [2]. It has been explored that 15% of cancers are produced by different oncogenic viruses such as Human T lymphotropic virus (HTLV), Kaposi's associated sarcoma virus (KSHV), Hepatitis B virus (HBV), and Human papillomavirus (HPV) [3]. In 1907 Italian Physicists first noticed that wart-like structures formed in women's cervical region. These warts are formed due to infection of one type of virus i.e. Human papillomavirus (HPV), later it was rediscovered in 1970. Several Human papillomaviruses (HPV) have been detected

as per their modalities of action on normal cells, but all HPV viruses like HPV - 11, 6, 42, 40, 44, 43, 54, and 53 have no capacity for strong infection, so they are low-risk types of viruses among all HPV. Another group of HPVS have strong infection ability and cell transformation capability like HPV - 16, 18, 39 and 45. They have some specific cell proliferation and transformation genes [4]. The significant death rate is decreased due to proper Pap smear screening and vaccination in patients [5]. The recovery rate is very poor in the case of the advanced stage due to insufficient action of chemotherapeutic drugs [6]. The survival rate of such patients for one year is only 10% - 20% after chemotherapy, due to the high progression rate of the cancer.

Another important fact is drug resistance, cervical cancer is caused by several multifactorial reasons, efflux of drugs,

drug detoxification, modulation of drug target mechanisms, irregular DNA damage mechanisms, changes of tumour microenvironment, and modulation of ROS generation are just for drug resistance [7]. So, there are some limitations of conventional chemotherapy. In this context strategy of chemotherapy, and radiotherapy should be changed for better improvement of cervical cancer. Conventional chemotherapeutics like Methotrexate, Cisplatin, Doxorubicin, DNA intercalating agents, molecular targeting synthetic compounds, anti-tubulin factors, and anti-metabolite compounds are not sufficient for cervical cancer treatment. Because of the poor progression of the disease and, the toxic effects of chemotherapeutic agents, another major issue is drug resistance. The overall scenario of anticancer chemotherapeutic treatment is not too much sufficient in some cases. Numerous anti-cancer drugs are identified and applied for treatment, but problems arise due to severe side effects on patients. Anti-cancer drugs have been categorized as per their mode of action and toxic effects. Bioactive phytochemicals play a major role in the treatment of different diseases like diabetes, heart disease, and anti-microbial activity [7]. Bioactive phytochemicals from different plants have been classified into different groups like terpenoids, di-terpenoids, flavonoids, alkaloids, and iso-pentenoid compounds. These are gaining much attention nowadays due to their high bioactivity and biocompatibility [8]. They are more effective and less hazardous to human health. Many Angiospermic plants have been documented as per their use as medicinal plants, since ancient times people have used different plants as traditional medicinal plants. According to current data more than 80,000 Angiospermic plants have been identified as medicinal plants by the International Union for Conservation of Nature (IUCN) and the World Wildlife Fund [9]. The application of biotechnology or molecular biology-based techniques like tissue culture or synthetic seed technology and molecular marker-based applications may improve the quantity of desired medicinal molecules and also improve plant growth. It should be implemented as part of the medicinal plant cultivation [10]. Some common medicinal plants which are found in nature have potent bioactive compounds used in different human diseases. Phytochemicals are obtained from different plant sources like natural fruits, vegetables cereals, beans, and many other sources. They have potent anticancer properties. For example, some flavonoid compounds like epigallocatechin, genistein, and epigallocatechin gallate (EGCG), are obtained from Soya products and have potential anticancer activity [11]. Since an early age Indian traditional medicines have been solely dependent on such types of plants like, *Andrographis paniculata*, *Citrus grandis*, *Cassia tora*, *Pterocarpus santalinus*, *Crocus Sativa*, *Mangifera indica*, *Artemisia afra* used in past time. Nowadays bioactive specific compounds are extracted from these plants and purified chemically for therapeutic purposes. Some extracted compounds have strong bioactive properties against cervical cancer [12]. Some important potent phytochemicals from natural resources and their role in several cancer cell deaths are given in Table 1 and chemical structures are given in Figure1A. In this study, we have investigated the major signalling pathways involved in cervical cancer cell apoptosis by searching different research articles published in past decades to till now. We have collected data after searching large numbers of articles by Google, Google Scholar, PubMed, and NCBI journals. The exact molecular cell death mechanism detection would enable us to search for new drugs for the treatment of cervical cancer in a proper way.

2. Overall scenario of cervical cancer

Cancer in the cervix region of females is very common all over the World, previous data has explored that cervical cancer is the third leading cancer in the World after breast cancer, colon cancer, and lung cancer. About 569,000 new cases of cervical cancer have been documented [13]. It has been further studied in 2018 that 4100 new cases of cervical cancer were found among 13000 new cases of cancer. Cervical cancer is the leading cause of death in all over the World. In 2018 that there are 8,400,000 new cases of female candidates were found, and among these 4,200,000 death incidents were recorded. Cervical cancer is responsible for 6.6%-7.5% morbidity and mortality of female patients [14]. German scientist Harald Zur Hausen first isolated the human papillomavirus (HPV) in 1974, later after ten years it was rediscovered by him that chronic infection in the cervical region was caused by HPV. He isolated two types of HPV viruses such as HPV16 and HPV18 [15]. Out of 200 cases, it has been shown that not all strains are too infective but some HPV (Human Papilloma Viruses) like HPV – 12 and HPV – 31 have been expressed are more infective than other types of strains of HPV viruses. There are 200 HPV viruses identified but among these, only 12 HPV viruses have been identified as carcinogenic type, HPV – 16 accounting for 60% reason for cervical cancer and HPV – 18 accounting for 10% carcinogenic in nature. It has been noticed that high-risk factors are responsible for infection of HPV viruses which can be estimated near about 248 – 435 times greater chances of carcinogenicity in infected females than in uninfected persons. About 80% of cases of cervical cancer infection take place by HPV virus. The preventive measures for cervical cancer through vaccination decreased from 1% to 1.9 % [16]. Cervical cancer develops in early adulthood and adolescence period. It would take 10 – 15 years to develop in the cancerous stage. Multiple drugs have developed against cervical cancer but a little bit of improvement is possible due to drug resistance. The main reason is the poor prognosis of the disease. Multiple drug resistance is the main reason for the poor improvement of cervical cancer patients.

3. Cell death and molecular events

According to the Nomenclature Committee on Cell Death (NCCD), there are three types of cell death apoptosis, necrosis, and mitotic catastrophe. Cellular death is not only restricted to the morphological pattern but also at the molecular level [17]. We know that apoptosis is a spontaneous cellular death process in all animal bodies, which eliminates extra unnecessary cells from the body. It is a self-suicidal process where multiple gene activities are responsible for the death of cells, and regulated by other factors in our body.

Unnecessary cells are hazardous in our body which causes pathophysiological changes in many dimensions. As a result, malignant cells are produced in our body. So, apoptosis is an active physiological process that is controlled by more than one signalling pathway. Several protein factors are also involved in this process. It is not only a biological phenomenon but also a controlled process where cell division is maintained in the animal body [18-19]. Failing of this

signalling process leads to uncontrolled cell division which later transforms into malignant cells. In this review work cervical cancer is highlighted which is developed due to failing normal signalling procedures, and later leads to malignancy. Lack of apoptosis is the major reason for uncontrolled cell division in cervical cancer. So, modulation of cell division by bioactive compounds would be the better way to combat cervical cancer bioactive compounds from different Indian traditional medicines would be given special attention, because of their capacity to alter the signalling pathway for apoptotic death of cervical cancer cells [5]. Human papillomavirus (HPV) contains some oncogenic proteins which play a crucial role in the development of cervical cancer [20]. The viral protein E6 interacts with p53 tumour suppressor genes and inactivates its functionality, as a result, uncontrolled proliferation of cervical cancer cells occurs. E6 protein is produced by the HPV virus, another E7 protein interacts with the p53 protein and Rb protein. Interaction between these two proteins causes uncontrolled growth of cervical cancer cells. Sex steroidal medicines play an important role in the progression of cervical cancer, it correlates with HPV proteins in the early stage [21]. In this context, awareness about cervical cancer is very much needed. So, the proper mechanism of cervical cancer cell proliferation and its exact signalling pathways should be explored. This can be induced by several phytochemicals (bioactive compounds), they directly induce apoptotic or autophagy cell death via different signalling pathways. The bioactivity of different phytochemicals should be toxic against malignant cells, otherwise, drugs extracted from plants would not be effective on cancer cells and should be tested in vitro and in vivo properly. From this point of view, apoptotic cell death of malignant cells is highly induced by various phytochemicals in different ways. We have thoroughly investigated and studied several updated research articles and analysed all data. Various pathways will be explored and summarized, which might be helpful for future researchers in this field. Understanding the molecular mechanistic pathways initiated by several phytochemicals would be helpful for therapeutic strategy.

4. Andrographolide compound switching on different signalling pathway

Andrographolide is a diterpenoid compound which is a very important bioactive compound obtained from *Andrographis paniculata*. It has been found that Andrographolide induced apoptosis via a death receptor-mediated pathway in several cancer cells [22]. TRAIL is an important pathway related to extrinsic apoptotic malignant cell death, but it doesn't induce normal cell apoptosis [23-24]. Applying this property many anti-cancer drugs have been established. Even TRAIL is the promising signalling pathway for the investigation of new weapons against cervical cancer. TRAIL – 1 & TRAIL – 2 binds with the receptor molecules on malignant cells where they change some conformation and molecular structure of receptor molecules on the cell surface. Even the TRAIL (Tumour related apoptosis-inducing ligand) pathway is related with Next time another molecular pathway in malignant cells like Fas-associated death domain (FAD) and caspase–8, caspase – 9 are activated which leads to apoptotic cell death of tumour cells. Sometimes TRAIL resistance property of a cell can be developed due to molecular changes of death receptor molecules as well as

over-expression of some anti-apoptotic factors like FLICE inhibitory factors, and XIAP factors which have inhibitory effects on the apoptosis program which are X – X-linked [25-26]. TRAIL resistance of the malignant cell is a very common phenomenon. Some effective compounds have been reported that can alter the resistance activity of malignant cells such as inhibitors of Myc protein, Raf kinase protein, and cyclin-dependent kinase inhibitors [27-28]. Andrographolide a diterpenoid compound proved its anti-inflammatory activity. It induces apoptosis in cervical cancer cells by activating TRAIL-mediated p53 gene expression which activates signalling death receptors DR 4 & DR 5 including C – Jun – NH 2 kinase (JNK) activation with reactive oxygen species (ROS) generation [29]. It is reported that upregulation of death receptors DR 4 and DR -5 would enable the cell to apoptotic death in the case of TRAIL-resistant cells. In this experiment, they have proved that treatment of TRAIL alone and treatment of TRAIL with bioactive phytochemicals diterpenoid compound Andrographolide obtained from *Andrographis paniculata* showed better results in cervical cancer cell HeLa. The combinatorial effect of andrographolide and TRAIL showed a synergistic effect on HeLa cells and another cell line [29]. For both TRAIL-resistant cells and TRAIL-sensitive cells the IC_{50} value of the Andrographolide compound was 60µM/ml. Andrographolide compound initiates caspase-dependent cell death, both caspase – 8 and Caspase – 3 were activated and cell death took place. A very interesting observation of this study was that both TRIL and Andrographolide alone have no significant effect on Caspase – 3 and caspase – 8 activations as shown in the schematic diagram (Figure 1B). However, the combination of both molecules altered the apoptotic cell death pathway by activating the Caspase cascade. Cleavage of Caspase -3 substrate PARP was a very important phenomenon for the death of TRAIL-resistant malignant cells.

5. Curcumin promotes cell death cascades and down-regulates proliferation factors of cervical cancer cells

Curcumin is one of the important bioactive compounds which can be obtained from *Curcuma longa*. Curcumin itself has much medicinal importance like relieving abdominal pain due to biliary dyskinesia [30]. It shows many bioactivities in different cases like antibacterial activity, anti-diabetic activity, and anti-inflammatory activity even, anti-tumorigenic activity is also noticed [31-33]. Curcumin has potent antioxidant properties and anti-tumorigenic properties experimentally have proved. It does not show any side effects on tumour-bearing mice (induced by radiation) [34]. We know that apoptosis is a multidimensional process regulated by many regulatory protein machineries.

Bcl2, and Bcl - xl all are anti-apoptotic proteins, they function as inhibitors of the apoptotic pathway Bcl2 protein over-expression increases cell viability by decreasing and withdrawing cytokine [35]. It has been reported previously by a few groups that curcumin-induced apoptosis in many malignant cells by activating molecular signalling pathways. Down-regulation of Bcl – xl, and Bcl2 was reported in Human Jakarta cell line T–cells where the Bcl–Xl gene has to be inhibited by curcumin treatment, another example can be expressed in Hut–78 another Human T-cell line, where these two anti-apoptotic genes, Bcl2 and Bcl–Xl were suppressed by curcumin compound. So, Curcumin not only inhibits the

T–cell line growth but also cervical cancer cell growth regulation can be effectively controlled by this unique compound. The unique compound has a very effective role in the alteration of singling pathways in the case of cervical cancer cell HeLa by downregulation of Bcl – 2 and Bcl – xl (Figure 2a), where it may promote the upregulation of apoptotic inducing proteins BAX and Bad by inhibiting up-regulation of Bcl–2 and Bcl – Xl [32]. The proteolytic enzyme caspase family plays a vital role in apoptosis, everybody knows that there are ten members in the caspase family. Among these members, Caspase – 3 directly executes the apoptotic cell death [36], caspase cascade is the main signaling pathway that overexpresses and directly regulates anti-apoptotic Bcl – 2 and Bcl–l factors as a result apoptosis occurs consequently. So, curcumin has a very efficient role in cervical cancer cell death (HeLa cell) via the caspase -3 mediated pathway Bcl–2 and Bcl–X1 inactivation by caspase was found in an earlier study. Curcumin-induced cell death in three cervical cancer cell lines i.e. HPV positive cells pre-treated with estradiol was investigated extensively [37]. Even it is proven that synergistic effects of the sequenced application of three compounds, curcumin Cisplatin and epigallocatechin-3-gallate (EGCG) have anti-proliferative activity on human ovarian cancer cell line A2780 and A2780^{cisR}, one is normal malignant ovarian cancer cell and another is Cisplatin resistance cell [38]. It has been known earlier that sex hormones increase the gene expression of HPV – 16, PCNA, and – 18 and help to transform the viruses in estrogen-sensitive cervix areas. Estrogen hormone has a significant role in cervical cancer progression. It alters the HPV virus and promotes oncogene activation as well as neoplastic progression in cervical carcinogenesis [39]. A high level of telomerase activity is shown in malignant cervical cells but short telomerase activity is observed in normal cells [40]. Curcumin decreases the activity of telomerase and Cyclin D1 in the case of estradiol-treated cells shown in the schematic diagram (Figure 2b). Even Curcumin counteracts the E6, PCNA, and E7 oncogene which is activated by estradiol, so estradiol-treated cervical cancer cells are unable to undergo apoptosis in the normal way. Curcumin plays a crucial role in this case and regulates all viral oncogenes [41]. As a result, regulation of both p53 and p73 tumor suppressor proteins was observed, a schematic diagram is given below (Figure 2c). Another study has explored the significant role of caspase – 3, and caspase – 9 activations after the treatment of Curcumin and down-regulation of all anti-apoptotic proteins like Cyclin – D1, iNOS, and COX – 2. In this context, curcumin was treated in three types of cervical cancer cell lines HeLa, SiHa, and CaSki. Overexpression of Cyclin - D1 protein and Hsp – 70 proteins are suppressed after the application of the Curcumin compound. Another important factor in apoptosis is the mitochondrial membrane protein Cytochrome – C releasing due to the induction of apoptosis by the Curcumin compound. After treatment of Curcumin overexpression of iNOS and COX – 2 were downregulated in all three cell lines HeLa, SiHa, and Ca Ski. This study very nicely described the ROS activity of cancer cell apoptosis after treating of Curcumin compound at a particular concentration of 10–50 µM/ml and showed variable results of the three mentioned cell lines (Fig.2a). So, there is a direct and indirect relationship between iNOS and COX – 2 down-regulation and ROS generation. NF – кB and viral protein E6, E7 positive cervical cancer cell responsible

for cell malignancy, so downregulation of NF – кB gene along with telomerase would be proposed signaling cascade for apoptotic cell death. Telomerase activity is an effective tool to detect carcinogenesis, it is a type of enzyme that is the combined form of RNA (hTR) and another protein component (hTERT). It is noticed that the telomerase activity of three cell lines reduced after Curcumin treatment. Another pathway is ERK/MAPK, which has a significant role in apoptosis progression [42]. Curcumin initiates MAPK pathway to activate cervical cancer cell apoptosis, it is also efficiently studied. As whole curcumin triggers apoptosis in cervical cancer cell lines which are related to ROS generation, COX–2 gene suppression, MAPK pathway activation, involvement of mitochondrial membrane potential breakdown, Cyclin – D1 suppression, and iNOS enzyme activation, all signaling pathways are directly and indirectly related with curcumin-initiated apoptosis in three cervical cancer cell line HeLa, SiHa and CaSki [41]. In the aforementioned three different cell lines curcumin acted in different modes such as downregulation of COX – 2, iNOS as well as cyclin D1 suppression. Recent studies have proved that curcumin induces autophagy cell death by switching on p21 and p53 proteins and ROS generation in the case of SiHa cervical cancer cells [43].

6. Comparative analysis of curcumin and other phytochemicals in cervical cancer cell death

Apigenin is another compound that is derived from plant extracts that have anti-tumorigenic effects on several malignant cells. There is a significant toxic effect of Apigenin & Curcumin on the Cervical cancer cell line, where both compounds showed Autophagy and apoptotic type of cell death in HeLa [44]. Both autophagy and apoptotic cell death are controlled by several signaling pathways (Fig. 3). Autophagy is a type of cell death where many factors are related like Atg12, Atg 5, and Beclin-1. Autophagy cell death depends on cellular conditions and environment where both apoptotic death and autophagy death are cross-talked with each other by some regulatory genes like Bif-1, BNIP-3, Bcl-2, Bcl-xL, and other genes like Atg5, Bif-1[45]. There is a cross-connection between autophagy & apoptotic cell death Bif–1 gene expression when the compound Curcumin and Apigenin were applied separately in different doses (Figure 3). Bf – 1 gene expression was high in Curcumin incubated HeLa cells in comparison with Apigenin-induced malignant cervical cancer cell death. Tumour necrotic factor (TNF) expression is another important phenomenon in autophagy cell death [46].

After incubation of cervical malignant cells by Apigenin and curcumin it was noticed that TNF - ∞ and FAS were upregulated and activated apoptotic cell death as well as autophagy cell death. We all know that Curcumin is a polyphenolic compound and has a potent anti-cancer effect on different malignant cells. This comparative study of two different phytochemicals can be helpful for the modulation of chemotherapy and better treatment for cervical cancer in the future. Commercial drugs and less toxic phytochemicals can be applied simultaneously for the improvement of the treatment of cervical cancer [47]. Combined effects of two different herbal products would be expected better results for cervical cancer cell death. Curcumin and Ellagic acid both can synergistically induce apoptosis in cervical cancer cell HeLa [48]. Ellagic acid is a naturally obtaining phenolic

compound, present in Grapes, Pomegranate, and Raspberry. Ellagic acid has special attention for its anticancer and anti-tumorigenic acid. Combined application of Ellagic acid and curcumin enhanced tumor suppression protein p53 restoration as well as downregulated p21 protein in cervical cancer cell HeLa. In general, viral oncoprotein E6 (HPV) degrades the p53 protein and leads to activating normal cervical cells into malignant cells. So, a previous study showed that restoration of p53 activity and suppression of the p21 gene leads to the pro-apoptotic protein BAX. DNA damage occurs in malignant cells due to ROS generation which later induces apoptotic cell death in Human cervical cancer cell HeLa.

7. Emodin as a potent bioactive anti-cancer compound enhances malignant cell death signal

Bioactive phytochemical Emodin is widely known for its several anti-cancer activities. It is a Chinese herbal compound obtained from *Polygonum Cuspidatum*. Emodin is one type of anthraquinone that has several multidimensional roles anti-tumor activity, anti-angiogenesis, and anti-proliferative activity in many malignant cells. The compound induces cell death in the lysosomal system in several malignant cells. Mitotic catastrophe can be modified by several anthraquinonoid groups of phytochemicals, where chromosomal breakdown takes place. Due to mitotic catastrophe poly-morphonuclear, giant cells, and micro-nucleated cells are shown. The same phenomenon was studied in cervical cancer cell HeLa after treatment of Emodin. All malignant cells lack control in the G1/S phase or cell cycle arrest phase G2/M phase (Figure 4a). It is observed that at $100\mu M$ concentration Emodin efficiently arrests the cell cycle at sub G2 level and induces the activity of cell cytoskeleton [49]. Emodin itself has potent anti-tumor activity properties, which inhibit the angiogenesis process by regulation of NF – kB-associated factors [50]. The synergistic effect of Curcumin and Emodin on HeLa and SiHa cells has a potent antitumor effect by downregulation of the TGF–β signaling pathway [51]. TGF – β signaling pathway is a complex cellular pathway that regulates cell proliferation and cell death by apoptosis and homeostasis is maintained in different dimensions. So, TGF–β signaling has special attention for making chemotherapeutic drugs. In the Human papillomavirus (HPV) TGF–β signaling plays a very crucial role in the progression of cervical cancer cell growth. Where TGF – β binds with TGF-β receptor II by the trans-phosphorylation method, which then activates the TGF-β receptor I by the trans-phosphorylation process in the - C terminal region. Later activates R - Smads proteins (Smads – 1 & Smads – 2). Smads protein then trans-locates into the nucleus and forms a hetero complex structure with Smads – 4 which triggers the activation of the TGF – β signaling pathway [52]. The TGF – β then regulates the pro-apoptotic and anti-apoptotic genes BAX and Bcl2 gene ratio. TGF – β regulates cell division at the G1 phase by suppressing CDCK and Cyclin – D 1 proteins. Even TGF – β also regulates cell migration and invasion as well as this factor alters another signaling pathway i.e. Wnt/β- catenin. Alteration of Wnt/β-catenin signaling pathway is responsible for cervical cancer cell neoplasia [53]. Increased level of TGF – β mRNA expression causes rapid proliferation of malignant cells, which is directly related to Wnt/β – Catenin signaling pathway. The above study showed that the combined effect

of Curcumin and Emodin had very adverse effects on cell survival (Fig. 4a). It is noted that different concentrations of both compounds regulate the TGF – β signaling and decrease the cell viability, mitochondrial membrane potential disruption takes place in both HeLa and SiHa cervical cancer cells. It has so far proved that the combined application of Curcumin and Emodin may be used for better results in cervical cancer therapy by targeting a particular molecular pathway. Emodin induces apoptosis in several malignant cells reported earlier. So, it also showed ovarian cancer, cervical cancer, and Human choriocarcinoma cell apoptosis in vitro like HO-8910, HeLa, and JAR. Another study showed that Emodin induces both autophagy and apoptotic cell death in human cervical malignant cells. In both cases signaling mechanism is different. In autophagy cell death they have studied that Beclin-1 and Atg12-Atg5 genes are downregulated and upregulation of MAP LC3 occurs. These activities are induced by the Emodin compound. Previously it has been proved that the Emodin compound inhibits metastatic growth of cells by downregulation of angiogenesis gene VEGF and VEGFR-2. Extracellular matrix (ECM) plays a crucial role in the invasion of malignant cells, but another enzyme matrix metalloproteinase (MMP) degrades the ECM and resists against invasiveness of malignant cells rapidly. After treatment of Emodin, the bioactive compound in malignant cells it has been shown that, matrix metalloproteinase - 9(MMP- 9) expression increases in many folds. Another important signaling pathway i.e. caspase – 9 dependent pathways have been noticed by them [49]. Mitochondrial membrane potential declined which reduces the ATP synthesis in malignant cells; it correlates with the caspase -9 activity in apoptotic cell death. Cell cycle arresting is another phenomenon that was caused by the Emodin compound in cervical cancer cells. Cyclin - D and Cyclin - E both gene expression inhibited by Emodin as a result apoptosis occurs. Another study showed that Glucose metabolism induces the proliferation of malignant cells, but it can be stopped by breaking the glucose metabolism pathway (Figure 4b). Emodin showed that glucose starvation inhibits cell proliferation and induces apoptosis by downregulation of glucose transporter protein GLUT – 1[54]. So, the Emodin compound can be an effective tool for the chemotherapeutic treatment of cervical cancer in the future. More unknown signaling pathways are yet to be explored.

8. Aloe-emodin (anthraquinone) induced cervical cancer cell death

Aloe – Emodin is an anthraquinone which can be obtained from many plants like *Rhamnus frangula*, *Rheum palmatum* and *Aloe barbadensis* [55]. The chemical name of the anthraquinone group of compounds its chemical name is 1,8-dihydroxy-3-hydroxymethyl-9,10- anthraquinone [56]. Anti-apoptotic nature of Aloe Emodin was reported in several cancer cell lines. In the case of tongue squamous cancer SCC-4 cells via a caspase-dependent pathway and by arresting cell cycle. It is known that Aloe – Emodin switches on p53, p21, BAX, Fas/Apo, and caspase 9, as well as caspase – 3 pathways and down-regulates the ERK – 1 pathway in bladder cancer cell and malignant melanoma cells [57-58]. Aloe – Emodin compound showed cytotoxicity against cervical cancer cell HeLa (Figure 5). Aloe – Emodin compound initiates mitotic catastrophe, where multi-nucleated cells were observed, even disrupted Golgi apparatus disorganized after its application. In mitotic

catastrophe cell size and shape are disrupted, where multi-nucleated cells and disorganization of microtubules also happen. Aloe – Emodin showed microtubules break down and disorganization of cells and promote cell death. The lysosomal compartment is involved in this type of cell death, which was proved by them earlier. In mitotic catastrophe, the cytostatic condition of Human cervical cancer cell HeLa changes drastically, where cytoskeleton, microtubules, and chromosomal breakdown lead to the anti-proliferative activity of cell cervical cancer cell HeLa (Figure 5). Mitotic catastrophe leads to caspase-dependent apoptotic cell death was also proved earlier. The compound is a very potent bioactive compound and can be used as a therapeutic agent for cervical cancer treatment. More investigation is needed about the aloe–emodin compound for future treatment of cervical cancer. The therapeutic potentiality of the compound Aloe Emodin would open a new trial for cervical cancer treatment.

9. 6-shogaol as a potent bioactive compound inhibits cervical cancer cell growth

Zingiber officinale is a traditional Indian medicinal plant that has many bioactive compounds responsible for anti-microbial, and anti-inflammatory and acts as an antioxidant as well as potent anti-proliferative for many malignant cells [59,60]. Mainly three types of compounds have been shown anti - have proliferative activity in cervical cancer cell HeLa, are 6-gingerol (6-G), and 6-dehydrogingerone (6-DG) these three compounds 6-shogaol (active phenolic compound) showed more potent anti-cancer activity against cervical cancer cell HeLa [61]. Recently it has explored a new trial of molecular signaling of this compound 6-Shogaol on human cervical cancer cell HeLa (Figure 6). The inactivation of thioredoxin reductase by the anti-cancer agent is a very important target to combat cervical cancer. Thioredoxin is ubiquitous in many cells, two types are thioredoxin is found i.e. Trx1/TrxR1 and Trx2/TrxR2 in the cytosol of the mammalian cell. Both thioredoxin (TrxR) and NADH are called the thioredoxin system. It has been studied that some important factors like Thioredoxin (Trx), thioredoxin reductase (TrxR) as well as NADPH, and the thioredoxin system are involved in many cellular activities and redox signaling pathways [62]. TrxR is also related to numerous diseases like cancer and neurodegenerative disease [63]. The TrxR is a selenoenzyme neurodegeneration that can be targeted for the treatment of cervical cancer. It has shown that HeLa cell death is oxidative stress-responsive cell death mediated by ROS after treatment of 6 - Shogaol. TrxR inhibited by 6 – shogaol and increased the cytotoxicity and ultimately apoptosis takes place. Another study has shown that 6 – Shogaol activates the PI3K/Akt/mTOR signaling pathway and ROS is produced after treatment of this phenolic compound in the HeLa cell line (Figure 6). It enhances cell cycle arrest at the G2/M phase in the different malignant cells. Even the xenograft mice model has proved that 6 – Shogaol inhibits tumor growth if it is applied to nude mice. Autophagy death is another phenomenon activated by the compound, it has been observed that expression levels of LC3-II, Beclin1, and reduction of p21 level in HeLa cells [64]. 6 – Shogaol is a potent inhibitor of thioredoxin reductase (TrxR). Recent studies have suggested that the combined effect of 6 – shogaol and salubrinal enhanced apoptosis in SMMC-7721 cells and SMMC-7721 tumor xenograft via ER stress-mediated PARP cleavage pathway [65]. So, 6 – Shogaol can be considered a future therapeutic agent. More investigation is needed to justify its beneficiary effects as a dietary food supplement to prevent cervical cancer.

10. Berberine an alkaloid compound induced cell death correlates with death receptor and mapk

Some Chinese herbs known as traditional Chinese medicine widely used in various fields to treat different types of diseases like diabetes, hyperlipidaemia, and cholesterol. Berberine is an Isoquinoline alkaloid compound that is used for the treatment of various kinds of disease. Berberine is also used in anti-inflammation and anti-cancer treatment. This alkaloid compound is derived from Chinese herbs used for hyperlipidemia, gastro problems, and cancer treatment in China [66]. The isoquinoline compound Berberine is obtained from the stems and bark of several plants like *Hydrastis Canadensis*, *Berberis vulgaris*, and *Coptis chinensis*, all are Chinese traditional herbs. Recently many researchers have explored the anti-cancer properties of Berberine and different modes of signaling pathways. There are many strong scientific reports about the anti-cancer activity of Berberine in different cell lines, even the compound also induces apoptotic cell death in cervical cancer cell HeLa by enhancing the activity of different types of chemotherapeutic drugs like Cisplatin compound. It is earlier known that ERK activation can modulate cell proliferation and it enhances the rapid growth of malignant cells [67]. Berberine compound induced cell death via caspase – 8 and caspase – 3 mediated pathways, along with mitochondrial involvement. Death receptor pathway was well studied in HeLa229 cervical cancer cells after treatment with the Berberine compound. It regulates different death receptor genes like Tumor Necrosis Factor Receptor Associated Factor 1 (TRAF-1), Fas, and FasL. It is known that mitogen-activated protein kinase plays a significant role in hepatocyte cell proliferation during mid-late (G1) phage [68]. The extrinsic pathway is directly involved in Berberine-induced cell death [69]. Activation of p38 Mitogen-activated protein kinase (MAPK) is very crucial for stress response cell survival and proliferation [70].

Activation of MAPK and JNK also induced by Berberine compound very effectively which may lead to p53 activation and apoptosis of HeLa229 cell, was proved by them. Very recently it has been detected that Berberine hydrochloride induces apoptosis in another cervical cancer cell line HeLa229. Both extrinsic and intrinsic pathway was involved in cell death, where COX2 was involved with the extrinsic pathway and Caspase – 3 and caspase – 9 were in the intrinsic pathway [71]. Activator protein and transcriptional protein play a crucial role in the progression and development of cervical cancer in humans. It is proved that Berberine-induced cervical cancer cell death blocks the E6 and E7 protein factors in the HPV (Figure 7) virus and simultaneously cell cycle arrest takes place in the cervical cancer cells [72]. Berberine may be applied against cervical cancer treatment, and understanding the molecular mechanism of Berberine-induced HeLa229 cell death can be considered for designing drugs in future treatment of such deadliest disease. More investigations are needed to understand the mechanistic pathway after treatment of the Berberine compound.

11. Combinatorial effect of berberine with chemotherapeutic compound on cervical cancer cells

Cisplatin compound and its derivatives have been widely used as an anti-cancer drug for a long time ago, but Cisplatin has many toxic effects on human health nephrotoxicity, neurotoxicity, and along with other complicated health issues can arise due to long-term use of such type drugs. Recently it has been found that the combined effect of chemotherapeutic drugs and phytochemicals would be a promising therapeutic strategy for cervical cancer treatment. However, the combined application of conventional chemotherapeutic drugs along with natural bioactive compounds can modulate and alter the adverse effects of chemotherapeutic drugs. So, the dose of drugs and chemo-resistance properties are two main factors in combating cervical cancer. Malignant cells easily develop chemoresistance against drugs, to overcome this problem combination therapy can be applied for the treatment of cervical cancer. Previously it has been known that Berberine has a toxic effect on Topoisomerase – I and Topoisomerase – II, it inhibits DNA synthesis in malignant cells. Even the chemo resistance of many drugs can be recovered by the combined effects of many phytochemicals along with conventional chemotherapeutic drugs. It also studied the combined effect of Berberine and Cisplatin on cervical cancer Cell HeLa. It has been noticed that a particular concentration of Berberine and Cisplatin i.e. 50µg/ml and 5µM enhances more than 54% of cell death. Mitochondrial membrane potential (MMP) breakdown where Bcl-2 and Bax3 expression was notified and release of cytochrome - C protein into the cytosol was recorded in this study. It was found that Bcl – 2 was downregulated and Bclxl was upregulated which are very significant study in apoptotic cell death. Changes in redox potential in the treated cell enhance ROS production was studied simultaneously with a combination of both compounds. So, the combined effect of Cisplatin and Berberine showed good results during apoptotic death of cervical cancer cell line HeLa. It can be applied in the preclinical and clinical stages, though more investigation is required about the combined effect of Berberine and Cisplatin in vivo conditions. Very recently it has been shown that the combined effects of Berberine compound and Matrine have anti-cancer effects on HeLa and SiHa cell lines. It has revealed both caspase - 3 and caspase – 9 activations as well as Bcl2 downregulation along with Bax upregulation are crucial for apoptosis in both cell lines [73].

12. Apigenin is a potent anti-cancer agent for cervical cancer cells and its signaling way

Apigenin is a flavonoid compound present in some fruits, vegetables, and sprouts. It has a magnificent role in many diseases like anti-inflammatory antioxidants, and anti-allergic, and also shows hepatoprotective nature. Even Apigenin has anti-cancer activity in different cell lines has been proved experimentally. Recent studies have shown that Apigenin inhibits the Raf/MEK/ERK pathway. It alters the ratio of BAX/Bcl – 2, ultimately inducing apoptosis in HeLa cells [74]. It suppressed transcription factor NF – kB and controlled cellular division in many dimensions in the case of epithelial cell apoptosis [75]. Apigenin treatment inhibits some malignant cells in vitro like colon cancer cells, breast cancer cells, and blood leukemic cells [76]. Apigenin compound induced apoptosis via p53 and arrest cell cycle in sub G1 phase in human neuroblastoma cells [77]. Earlier it

was found that Apigenin induces human diploid fibroblasts cell death by arresting the cell cycle in G1-level fibroblasts [78]. The Apigenin induces apoptosis in cervical cancer cell death HeLa dependent on p53 and p21. It has proved that at 37µM concentration Apigenin induces apoptosis in the cervical cancer cell (HeLa) by arresting the cell cycle at the G1 level (68.1% cells were arrested), and p21 gene expression is very important for cell cycle arrest at the G1 level. Even Apigenin strongly induces the upregulation of the p21/WAF pathway at the low level of p53 expression. Thus, p21 and p53 gene upregulation takes place at 37µM concentration. Another signaling pathway i.e. Fas/APO-1 plays a very significant role in apoptosis, p53 induces cell surface receptor FAS and enhances cell death very sharply [79]. It is noted that Apigenin enhanced the up-regulation of Fas expression and activated the p53 gene very actively. So, Apigenin can be considered as a potent phyto chemical for the treatment of cervical cancer.

13. Genistein an isoflavonoid compound activates several signaling pathways in apoptosis

Polyphenolic compound isoflavonoid compound has beneficiary effects on cancer treatment. It is reported that a compound isolated from Soya has induced apoptotic cell death in several malignant cells via different signaling pathways [80]. Meanwhile, it has been proved that Genistein has good anticancer properties, which inhibit tongue carcinoma cell lines in vitro. It has been noticed that Genistein inhibits cell proliferation by inhibiting vitro nectin and surviving protein. Both are upregulated in tongue cancer cells [81]. Kim SH *et al* (2009) have studied that Genistein compound induced apoptosis in the cervical cancer cell (HeLa, CaSki, and C33A). They studied the anti-proliferative activity of three cervical cancer cells and noticed that at a particular dose (60µg/ml) Genistein compound inhibited up to 45% cell growth in vitro condition in the case of HeLa cells. But other cells like CaSki, and C33A showed a little bit positive result than compared with HeLa.

In apoptosis of cervical cancer cells after treatment of Genistein compound both extrinsic and intrinsic caspase pathways (Caspase -3, Caspase – 8 & Caspase –9) are involved [82]. Tumor necrotic factor and receptor protein as well as Fas/CD95 receptor activate the extrinsic pathway by activating caspase - 8, reported earlier [83]. In both extrinsic and intrinsic pathways DNA damage takes place, which is activated by caspase -3 and caspase - 9 expression. It had explored that the Bax/Bcl2 ratio increased and PARP cleavage took place which is an important phenomenon for apoptotic death by Genistein treatment. The Bax/Bcl2 ratio increased simultaneously Bid decreased after treatment of Genistein, which indicates a cross-talk between intrinsic and extrinsic pathway. Previously it is reported further that Genistein induced apoptosis in HeLa cells by altering the activity of the TIMP1 and Matrix metalloproteinase (MMP – 9) by arresting the cell cycle at the G2 level [84]. Endoplasmic stress response apoptosis is another important factor responsible for malignant cell death. Several drugs have been identified that lead to apoptosis via endoplasmic response way [85]. Endoplasmic reticulum stress-mediated several diseases, as a result, disturbed ER shows unfolded protein response (UPR) to maintain the calcium homeostasis. Misfolded proteins are produced during ER stress conditions, to maintain and restore normal conditions some

immunoglobin protein factors, such as GRP78, and IRE1α (inositol-requiring transmembrane kinase and endonuclease 1α), are produced that can be helpful for the restoration of ER response activity [86-87]. Yang *et.al* showed that cervical cancer cell apoptosis induced by Genistein is ER stress-mediated. After Genistein application on HeLa cells, some ER stress protein like GRP78 is elevated; it is the molecular marker of ER stress. Genistein also induces GRP78 and CHOP protein expression related to ER stress-mediated HeLa cell apoptosis. Genistein has an epigenetic natural modifier role on human cervical cancer cell HeLa where it targets several genes like DNA- Methyl transferase (DNMTs) as well as Histone deacetylases (HDACs). In both cases, Genistein inhibits the activation of DNMTs and HDACs [88]. Liu et al, (2019) reported that the combined effect of Cisplatin and Genistein reduced the dose of Cisplatin and activated many apoptotic-related factors like P53 expression, pro caspase and cleaved caspase expression, many times after both HeLa and CasKi cells (Figure 9). The combined effects of Cisplatin and Genistein in so-called C+G groups have a synergistic effect on cervical cancer cell death [89]. So, Genistein can be effective for the treatment of cervical cancer and it showed a multi-dimensional signaling pathway during cervical cancer cell death.

14. Anti-proliferative activity of garlic compound on cervical cancer cell

Garlic has many health benefits anti-inflammatory properties, anti-bacterial properties even, and lipidemia properties. Some chemicals that have been isolated from garlic show anti-proliferative activity in different cancer cells like colon cancer, and breast cancer [90-91]. A major bioactive compound isolated from garlic is organosulfur, di-allyl disulfide (DADS), and S-allyl mercapto cysteine (SAMC) showed anti-proliferative activity in different cell lines. It has been proved that DADS induces apoptosis in cervical cancer after heavy carbon ion radiation (High LET). DADS have potential radio sensitization power which promotes pro-apoptotic factors Tap73 as well as anti-apoptotic factors like ΔNp73 along with down-regulation proteins like FASLG, and APAF1 [92]. Allicin compound obtained from Garlic extract is transformed into Allicin by an enzymatic activity. Allicin compound induced anti-proliferative activity and autophagy cell death in several cancer cell lines [93]. Heat-treated extract of Garlic has shown antiproliferative activity in cervical cancer cells by autophagy cell death. Further, it has been reported that cell death depends on the storage procedure of garlic extracts [94]. Long-time heating of garlic may decrease the bioactivity of extracts i.e. cell proliferation properties may be lost. So, overheating might lose the anti-proliferative activity of the compound. Allicin compounds present in garlic should be freshly prepared. It was reported earlier that Allicin inhibits cell growth by activating different signaling pathways [95]. Again, it is found that Allicin inhibits the growth of cervical cancer cells by inhibiting NRF2 expression [96]. NRF2 is an important signaling molecule that maintains normal homeostasis by overcoming oxidative stress in adverse oxidative conditions. It has been reported that NRF2 (nuclear factor erythroid 2-related factor) binds with the ARE (anti-oxidant Response element) and regulates the balance of oxidant [97-98]. NRF2 can be over-expressed and suppress the apoptosis of malignant cells as a result multiple tumor

development takes place. It has been reported that Allicin can suppress cervical cancer cell (SiHa) proliferation by inhibiting NRF2 expression. NRF2 expression enables cell chemoprotection, relief from oxidative stress, and radiotherapy protection [99]. Recent studies have revealed that organosulphur compounds from garlic showed anti-proliferative activity in cervical cancer cell Caski (cervical cancer cells) by down-regulating CDK4 and up-regulating CDK inhibitor like p21WAF1/CIP1 and p27KIP1 cervical cancer cell Caski. Even the organosulphur compound of garlic extract inhibits the viral oncogene E6 and E7 and acts as potent anti-cancer activity in the case of cervical cancer cell Caski [100]. Allicin compound shows multi-dimensional effects on various cancer cell lines.

15. Saikosaponin potent bioactive compound effects on cervical cancer cell

Saikosaponin, a triterpenoid glycoside compound obtained from the Chinese herbs *Bupleurum falcatum*, has many therapeutic efficacies. Saikosaponin shows many bioactivities and combat against disease like anti-inflammatory, antioxidant, and neuromodulator agents [101]. In a recent study there are 100 terpenoid saponin compounds have been identified, among these Saikosaponin A, Saikosaponin B, Saikosaponin B2, and Saikosaponin C have been considered pharmacologically active compounds for various diseases [102]. The pharmacological activities and mechanism of the anti-proliferative nature of Saikosaponin in many cell lines like lung cancer, colon cancer, and in hepatocellular carcinoma cells have been investigated extensively [103]. Recently it has been proved that, Saikosaponin-A induced apoptosis in cervical cancer cell HeLa mitochondria-dependent and ER (endoplasmic reticulum) dependent pathway in vitro and vivo in xenograft nude mice model [104]. Saikosaponin A is very effective for apoptosis in several malignant cell lines.

It induces breast cancer cell death by suppressing CXCR4 and induces DNA damage as well as caspase pathway activation (Caspase – 4) in colon cancer cells [105]. In cervical cancer HeLa showed drastic changes after the application of Saikosaponin A, where it initiates both extrinsic and intrinsic pathways by the breakdown of mitochondrial membrane potential (MMP), ROS generation is another hallmark of apoptosis. After the application of Saikosaponin A, it enhances ROS production. Bcl2 and Bax's ratio is an important clue of cell death, where Bcl2 is downregulated and Bax is upregulated, in both cases Saikosaponin played a very active role in the enhancement of cell death. We know that the endoplasmic reticulum is an important unfolded organelle for protein synthesis. During stress conditions homeostasis is maintained when unfolded organelle proteins are formed so; that ER initiates cell survival states through unfolded protein response (UPR). Thus, UPR failure leads to GRP78 activation and promotes ER stress condition which activates apoptosis. In cervical cancer cells, HeLa ER stress triggers to promote apoptosis. Tumor growth decreased in vivo experimental conditions in xenografted nude mice model. PI3K/Akt signaling activates cell proliferation and invasion, drug resistance, and tumorigenic activity in several malignant cells, as reported earlier. Saikosaponin compound-initiated cell death activated through intrinsic, extrinsic, and mitochondria-mediated pathways, endoplasmic reticulum stress as well as

downregulation of the PI3K/Akt signaling pathway. For this reason, Saikosaponin A can be considered a promising anti-cancer compound if its targeting molecular pathway is explored properly. In the future, it is expected that Saikosaponin A will be used as an effective drug for chemotherapy in cervical cancer treatment.

16. Eugenol isolated from clove (syzygium aromaticum) is a potent anti-cancer agent

Phytochemicals of clove are considered very potent anticancer compounds to combat cervical cancer cell proliferation. Clove contains several compounds like phenolic compounds, sesquiterpenes monoterpenes, and hydrocarbons. Eugenol has many beneficiary roles in human health. It was reported earlier that eugenol (4-allyl-2-methoxyphenol) is used in different fields like perfume making, and used as conventional medicine in Asian countries as an anti-bacterial agent, pain reliever, and antiseptic agent [106]. In the US country, Eugenol compound is used in toothpaste, even though it acts as an anti-viral compound [107-108]. At low concentrations, eugenol acts as an anti-oxidant agent, but at high concentrations, it causes cell damage and DNA breakdown takes place in human fibroblast cells [109]. A dozen reports have been published about the antiproliferative effects of eugenol compound in malignant cell lines like human melanoma G361, human leukemia cells (HL60), HT-29 cells (colon cancer), and breast cancer MCF7 cells, where eugenol induces cell death in a different mode of signaling cascades. In the case of human melanoma cell G361 apoptosis took place via a caspase-dependent pathway, involvement of mitochondria, and p53 expression [110]. Combined effects of Eugenol along with Cisplatin compound and X-ray radiation in cervical cancer cells have been studied by several groups. The combinatorial activity of eugenol and Cisplatin showed better results in HeLa cells which was synergistic and modified the activity of Cisplatin. Detailed signaling pathways have been explored and showed that decrease in proliferation and an increase in LDH activity. Even though mitochondrial membrane potential breaks down, caspase -3 activities are upregulated. Simultaneously it has been observed that COX – 2 proteins, Bcl – 2, IL - 1β was down-regulated and a synergistic effect of eugenol was noticed in Cisplatin and X-ray radiation-induced cells [111]. The previous report of Eugenol in apoptotic cell death in HeLa by activating caspase -3 and -9 pathway, mitochondrial involvement, Bcl2, and Bax regulation were well documented [112]. Another interesting report on synergism in more than two drugs has been reported, where it is reported that myricetin (MYR), methyl eugenol (MEG), and Cisplatin (CP) have a synergistic effect on cervical cancer cell HeLa [113]. The major observation was explored about the prevalence of cervical cancer related to epithelial-mesenchymal transition (EMT), where metastasis of cervical cancer cells and their invasiveness rapidly increased after a certain time [114]. In other words, the signaling is involved with Snail – 1, which is a zinc finger transcriptional factor – 1 that up-regulates the PI3K/Akt signaling pathway. As a result, EMT is increased and epithelial cells are modified to mesenchymal cells, loss of apoptotic cell death, invasion, extracellular matrix protein production, and resistance against cell death [115]. Down expression of E-cadherin protein is observed when EMT is increased and as a result, invasion of cells also increases [116]. Eugenol dramatically inhibits the EMT binding with

snail – 1 E – cadherin expression was regulated at an optimum level of eugenol compound (100μM) vimentin protein expression disappeared. Mesenchymal marker proteins like fibronectin and vimentin are attached to EMT. As a result, MMP9 another matrix metalloproteinase enzyme activates after the incubation of HeLa cells by eugenol. They also observed that EMT expression disappeared at a 200μM concentration of eugenol. So, eugenol had effectively controlled the EMT and Snail -1 expression as a result E – cadherin expression was increased. The invasion and migration of HeLa cells were controlled in well fashion. These signaling pathways can be targeted in cervical cancer treatment. Their study would open new doors in the future for the treatment of cervical cancer [117].

17. Fisetin is a flavonoid compound that has anti-proliferative activity in cervical cancer cell

Many flavonoid compounds have bioactivity reported earlier, basically, our common diets contain various types of phytochemicals and Polyphenolic compounds. Fisetin compound (3′,4′,7-Tetrahydroxyflavone) is present in many fruits and vegetables like strawberries, apples, onions, etc. even though it shows strong anti-oxidant activities, and anti-inflammatory activities [118]. Fisetin is a potent anti-cancer agent that has been proved by several researchers. Another important caspase-mediated pathway was reported in human non-small lung cancer cell apoptosis induced by Fisetin [119]. Signaling pathways in apoptosis induced by Fisetin have been revealed in different human malignant cell lines like Huh-7 and SK-Hep1. In all cases, Caspase-3–3 expressions, p53 expression, and mitochondrial membrane potential breaks down were noticed, after treatment with the Fisetin compound [120].

Even Fisetin plays a crucial role in the case of anti-inflammatory activity, which alters the radio-resistance properties of liver cancer cells by ER stress pathway [121]. Ying TH et al., 2012 explored that Fisetin has induced apoptosis in cervical cancer cell HeLa by caspase–3 and caspase–8 activation mediated by ERK1/2. They applied the Fisetin compound on several malignant cells like HeLa, A549, RL95-2, MDA-MB-231, U2OS, HepG2, and SW480. Among these cell lines, Fisetin inhibits HeLa cell proliferation and promotes apoptosis more rapidly than other malignant cells. The inhibitory effect of Fisetin is related to caspase- 8 and caspase - 3 but not related to caspase – 9. It has been reported that the Fisetin compound inhibits non-small lung cancer cells (NCI-H460) by downregulation of Bcl2-related X2 protein and reduces the B cell lymphoma-2. In this study, the authors have studied vastly about major signaling pathways in the HeLa cell line initiated by Fisetin. Previously it was known that mitogen-activated protein-kinase play (MAPK) a vital role in cell proliferation, cellular differentiation, and cell death, so it is a multifunctional signaling mediator [122]. Even it was found that Fisetin-induced apoptosis in HeLa cells was stress-dependent, where ERK1/2 expression occurred and mediated cell death by the activation of caspase (8 & 3), this was proved by them using inhibitors against ERK1/2 inhibitor (PD98059), JNK1/2 inhibitor (SP600125) and p38 inhibitor (SB203580). Among these three only ERK1/2 inhibitors (PD98059) attenuated cell death by Fisetin, where cells were pre-treated with inhibitors. Even transfected HeLa cells by DN-ERK1/2 reduced the expression of ERK1/2 and cell death is inhibited. So, the

ERK1/2 pathway plays a vital role in apoptotic cell death caused by Fisetin flavonoid [123-124]. Even in the xenograft nude mice model, Fisetin showed dramatic changes in tumor growth in size and shape. So, the in vivo model before the mentioned group has got better results and proved its anti-tumorigenic activity also. Recent studies showed Fisetin induces apoptosis by arresting the cell cycle at sub G2/M level, it modulates pro-apoptotic and anti-apoptotic gene expression like Bad, Bax, Bid & APAF1(pro-apoptotic gene) and Bcl-2, Livin/BIRC7, BIRC8, MCL-1, XIAP/BIRC4, clap-2/BIRC3 all are anti-apoptotic gene). It has been observed that downregulation of AKT and MAPK signaling path and enhanced apoptosis in HeLa cells in vitro [125]. Pro-inflammatory and anti-inflammatory cytokines in oxidative stress condition of cells by up-regulating superoxide dismutase, glutathione peroxidase, and superoxide dismutase. On the other hand, pro and anti-inflammatory genes are downregulated by the application of Fisetin in radio-resistant liver cancer cells. The recent investigation explored the role of the Fisetin compound in cervical cancer cell apoptosis. So, the anti-inflammatory, anti-oxidant properties of the Fisetin compound can be an effective therapeutic regime for better results in cervical cancer treatment. It is previously described that Fisetin induced cell death by arresting cell cycle G2/M level. Some molecular expressions of genes like CCNB1, CCNB2, CCNE2, CDK2, CDK4, and TERT are correlated with G2/M level cell cycle arrest [126]. It is again reported that all these genes are downregulated after Fisetin treatment in cells and cell cycle arrest at the G2/M level. Elevation of another gene like PTPRR, FOXO1 & and FOXO2 indicates anti-survival activity of cells. Incubation by Fisetin compound on cervical cancer cell HeLa above mentioned genes was upregulated and leads to apoptosis of cervical cancer cell. Combinatorial effects of several anti-tumor agents and bioactive compounds have been studied extensively. The combined effects of both agents give a new molecular trail and provide better therapeutic efficiency of drugs. In case of Fisetin also showed a new strategy for cervical cancer cell death with another anti-cancer agent multi-kinase oral drug sorafenib. Sorafenib, the multi-kinase inhibitor drug has anti-tumorigenic and anti-angiogenic activity as well as promotes some pro-apoptotic and anti-apoptotic inhibitors [127]. It is shown that the combined activity of Fisetin and Sorafenib induced HeLa cell death by death receptor (DR4), mitochondria, and caspase -8 & -3 mediated pathways [128]. The researchers have investigated the following molecular pathways. Combined application of sorafenib and Fisetin activates caspase – 8 and caspase – 3, as well as mitochondrial membrane potential breakdown, occurs, increased level of Bax/Bcl2 was observed which is a hallmark of apoptosis in HeLa cells. PARP cleavage is another phenomenon they have observed after simultaneous application. Sorafenib is a potent anti-angiogenic multi-kinase agent which inhibits different types of growth factors like platelet-derived growth factor receptors, and vascular endothelial growth factor [129]. Such type of example in the case of lung cancer cells combined effect of sorafenib and a derivative of benzofuran showed a synergistic effect [130]. So, combination therapy is more advantageous than single therapy. It has been reported that both Fisetin and sorafenib have an anti-tumor effect on xenograft nude mice models. Sorafenib and Fisetin induced apoptosis in HeLa cells by DR4 and DR5 activation, because DR5 plays a crucial role in

anti-tumorigenic activity, which has been proved by authors by pan-caspase inhibitors (Z-VAD-FMK and siDR5). Inhibitors block the synergistic activity of both compounds; as a result, apoptosis does not occur. There are many examples of combined effects of sorafenib and Fisetin, such as BRAF mutated melanoma cells showing down regulation of N–cadherin protein Vimentin, and fibronectin anti-apoptotic proteins. Which was proved both vivo and in vitro mouse xenograft tumor models [131]. In case of combined effect, HeLa cell undergoes apoptosis by DR5 activation and mitochondrial membrane potential break down by releasing cytochrome - C into the cytosol. So, the combined effect of Fisetin and sorafenib would be very effective for cervical cancer treatment.

18. Oleanolic acid is a potent anti-proliferative bioactive compound

Oleanolic acid is a natural pentacyclic triterpenoid compound that has many anti-cancer activities. It has the best inhibitory effects on EGFR – LTC kinase enzymatic activity. Oleanolic acid has many biological activities such as anti-oxidant, anti-inflammatory as well as anti-tumorigenic activities [132]. Oleanolic acid is present in the leaves, and fruits of many plants. It is a potent anti-cancer activity in different malignant cells, like leukemia cells, and non-small lung cancer cells. Oleic acid decreases oxidative damage of human breast cancer cells (MCF10A, MCF – 7, MD – MB – 231) and acts as a chemo-protective agent for breast cancer cells [133]. Even Gefitinib (used in non-small lung cancer treatment) resistant non-small lung cancer cell growth can be inhibited by oleanolic acid [134].

A new study has shown that oleanolic acid induced iron-dependent cell death i.e. ferroptosis in HeLa cells. Ferroptosis is a process where another signaling pathway is activated to initiate malignant cell death like kidney and liver cancer. Ferroptosis is regulated by the ACSL4 gene, which is a member of the long-chain acyl-CoA synthase long chain. ACSL4 is a ligase that is helpful for the synthesis of PUFA-PLs. Elevation level of ACSL4 leads to iron-dependent cell death i.e. ferroptosis in malignant cell death by accumulating lipid intermediates compounds in malignant cells which is a hallmark of ferroptosis. Oleanolic acid inhibits cervical cancer cell death by reducing ACSL4 gene expression and elevating the lipid peroxidation process. Another important phenomenon has been explored that GPX4 and GSH increase the lipid peroxidation process in the cell, after oleanolic acid incorporation into the HeLa cell activity of GPX4 and GSH drop-down, as a result, ferroptosis takes place. In lipid peroxidation iron ions Fe^{3+} and Fe^{2+} ion play an important role where free radicals like ROS, and hydroxide ions are generated. Transferrin is important for the delivery of Fe^{3+} into the cells, it binds with TfR1 on cell surface molecule and incorporates Fe^{3+} ion through DMT or divalent metal transporter – 1. Fe^{2+} is converted into Fe^{3+} during the peroxidation process and accumulation of iron in the cells occurs. Ferritin is responsible for iron accumulation; it is reported that it has two chains one is a ferritin heavy chain (FTH 1) and another ferritin light chain (FTL1). The ferritin heavy chain is responsible for the (FTH1) conversion of Fe^{2+} to Fe^{3+} and is stored in the nucleus by ferritin peroxidase reaction. It is reported by the authors that ferritin heavy chain 1(FTH1) is decreased and upregulation of ACSL4 takes place as a result of cervical cancer HeLa cell death happened by

ferroptosis method. Even it was also proved in the xenograft nude mice model that the accumulation of Fe3+ in the tumor of mice was higher than in control mice. Oleanolic acid treatment significantly reduced the tumor size. ROS generation after treatment of oleanolic acid was increased significantly in the xenograft tumor nude mice model. So, it can be concluded that oleanolic acid-induced HeLa cell death (Ferroptosis) is dependent on ACSL4 expression.
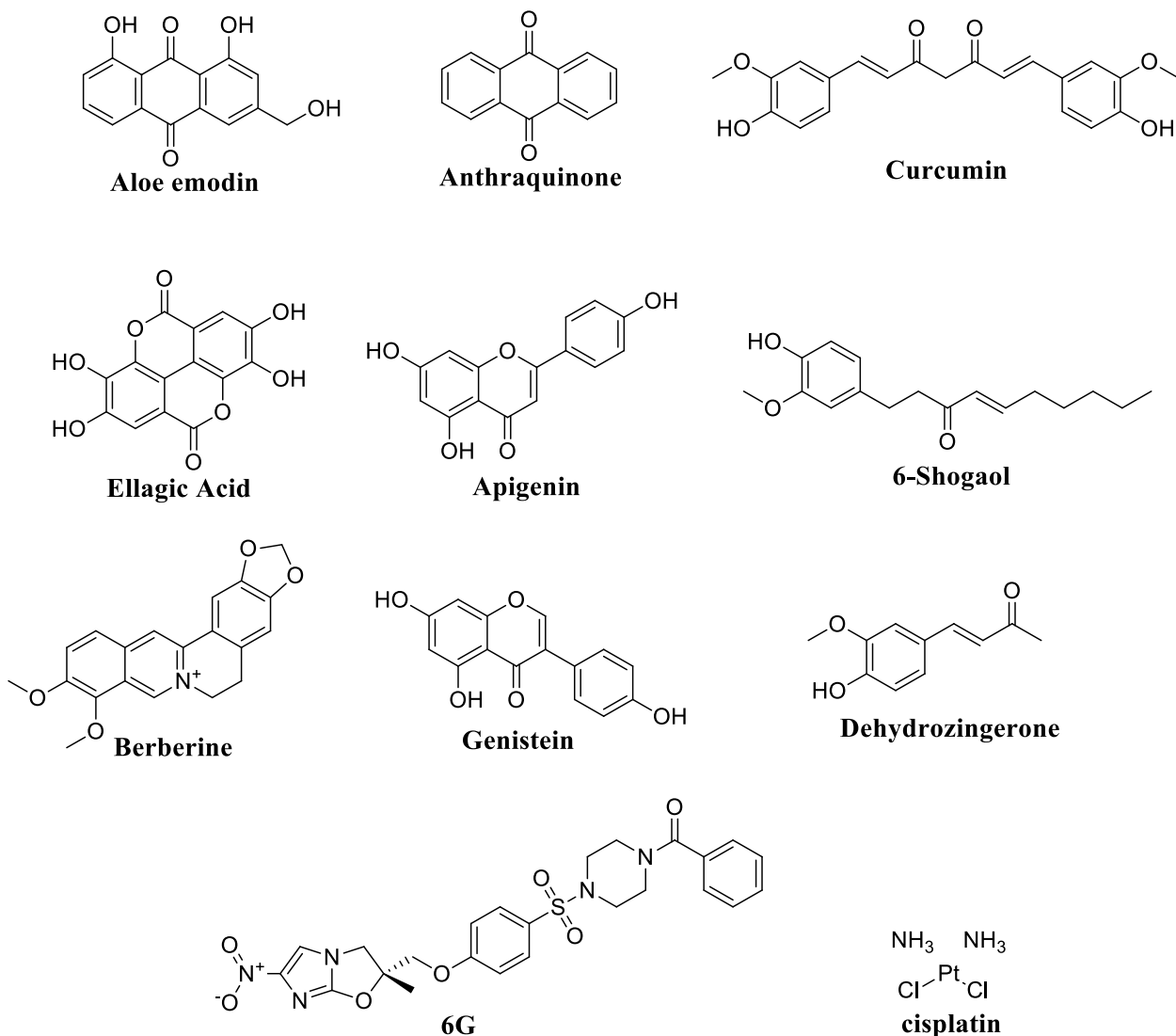

Figure 1A: Chemical Structures of some important phytochemicals and anti-cancer compound Cisplatin which are found as apoptotic agents in cervical cancer cells, the mechanism of apoptosis is discussed in detail in the main manuscript.
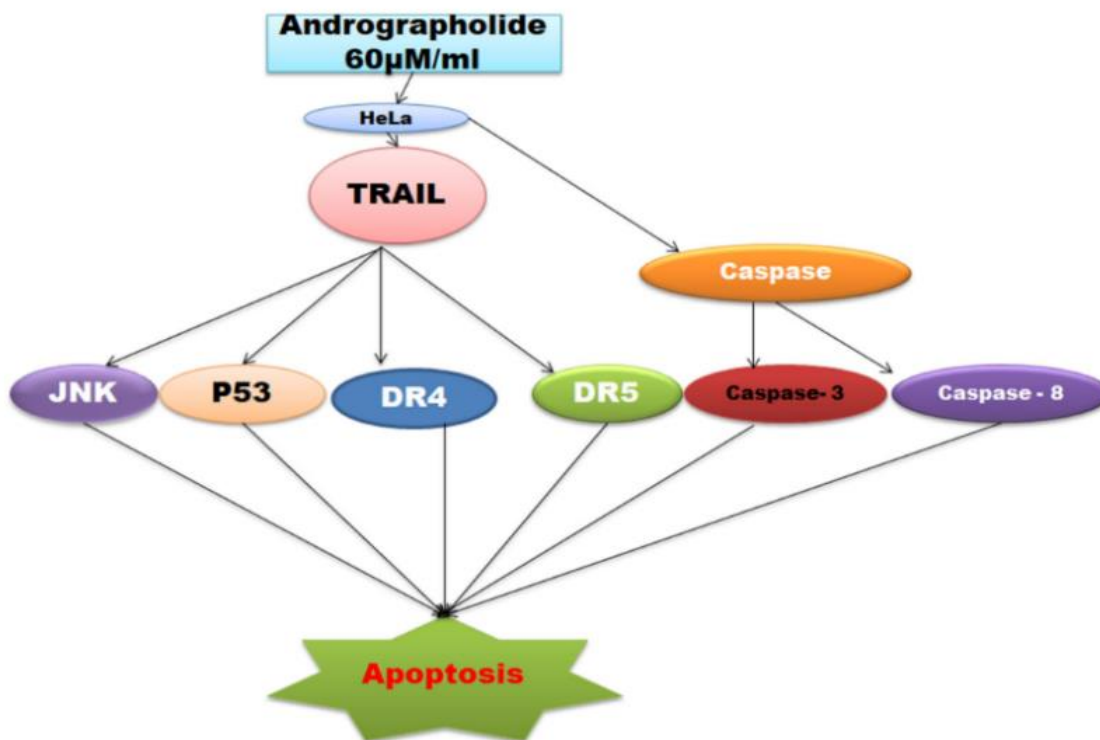
Figure 1B: The above schematic flow diagram shows the activation of TRAIL as well as death receptor proteins along with Caspase -3 and – 9 which is initiated by Andrographolide (60μM/ml) on HeLa cell for apoptosis.
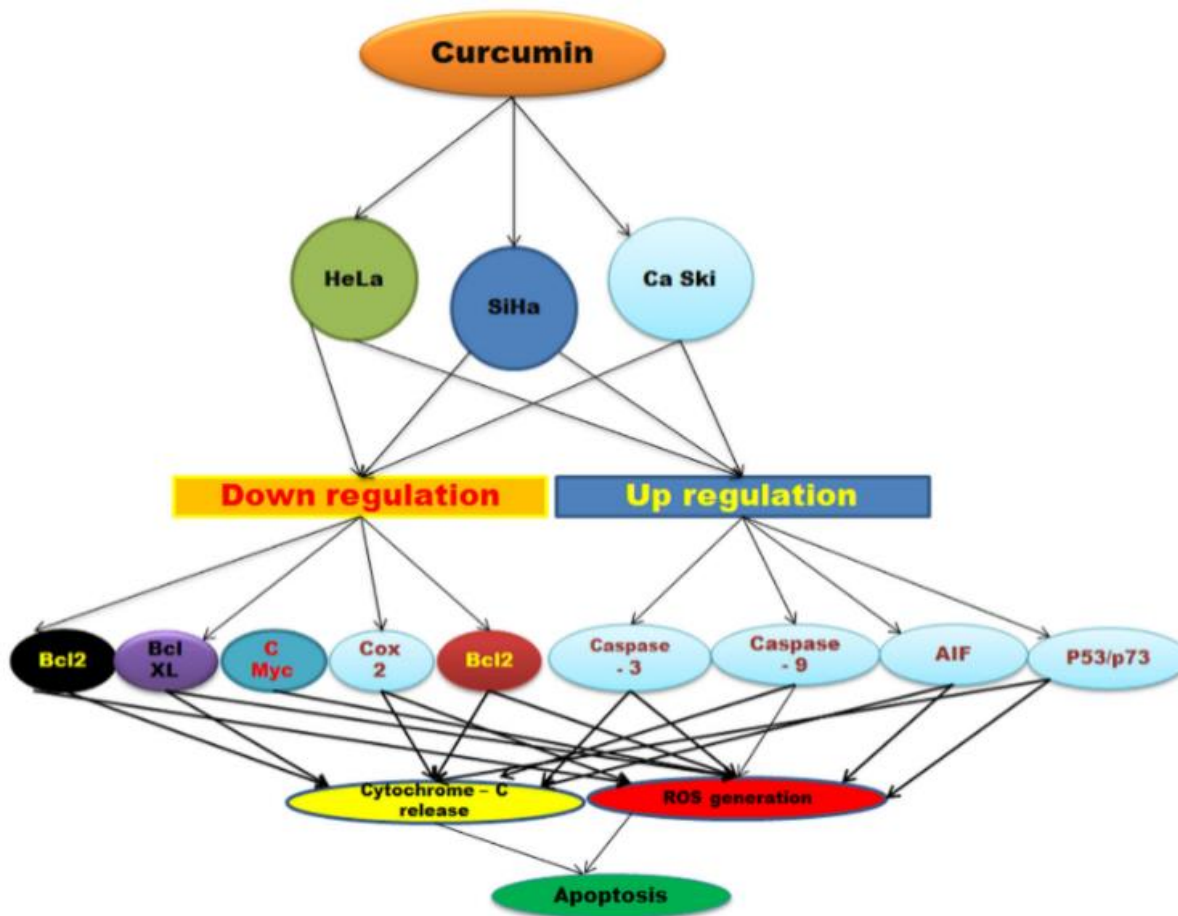

Figure 2a: The above schematic diagram shows the proposed pathway of apoptotic cell death in three types of cervical cancer cell lines (HeLa, SiHa and CaSki). ROS generation and Cytochrome – c release are the key factors of cell death [37].
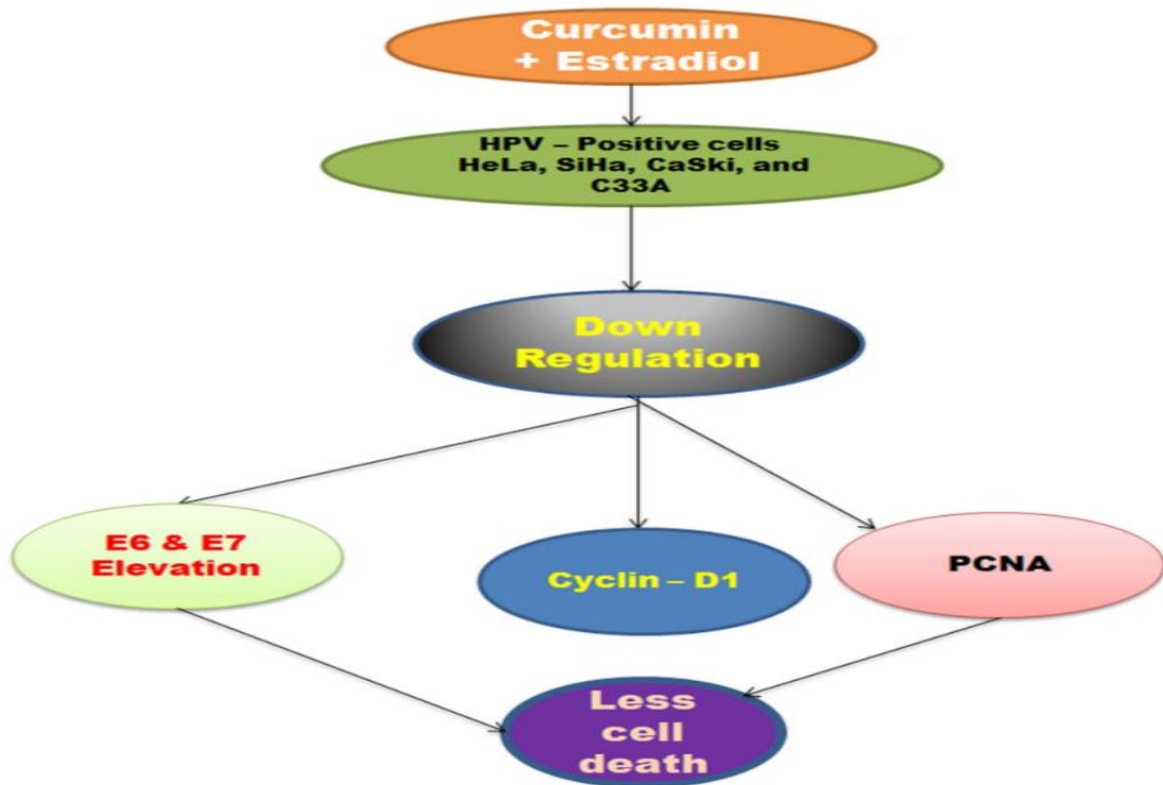
Figure 2b: Molecular mechanism curcumin and estradiol-initiated cell death in cervical in HPV positive cell line (HeLa, SiHa, CaSki and C33A). The schematic diagram shows that E6 & E7as well as PCNA oncogene down regulated after treatment of curcumin in estradiol treated cells.
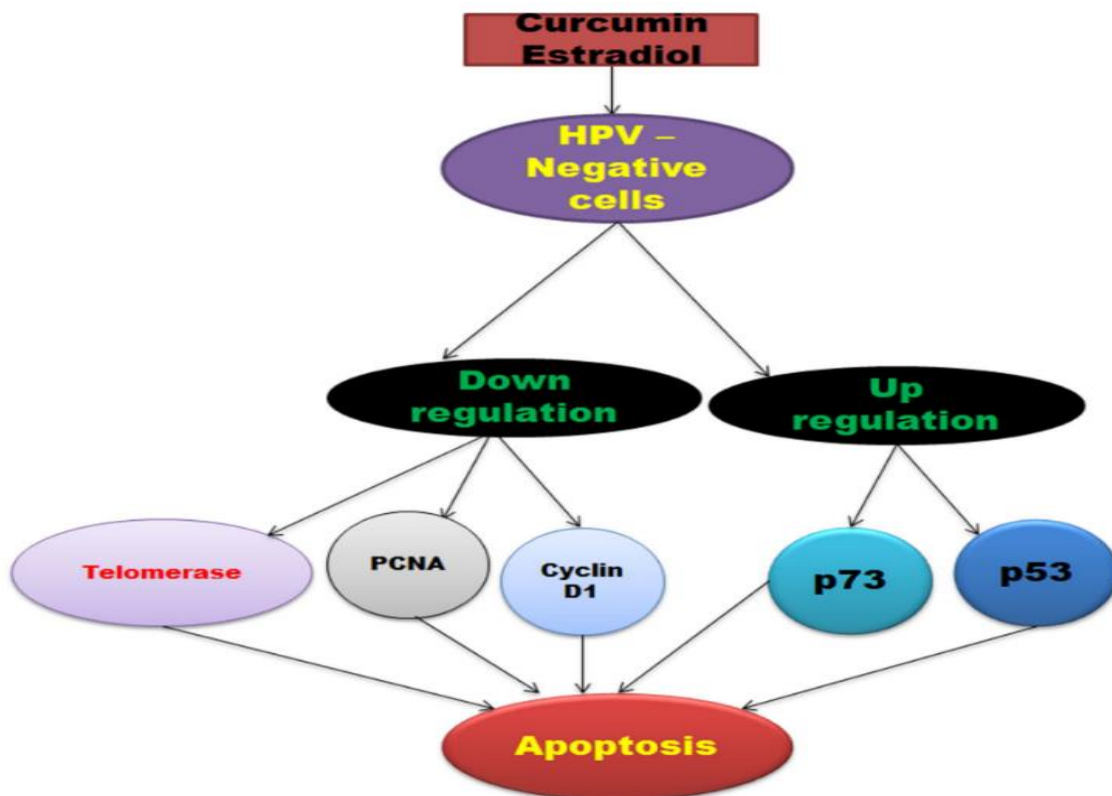

Figure 2c : In case of HPV negative cervical cancer cells (curcumin and estradiol treated) reduces telomerase, PCNA and cyclin – D1. Simultaneously p73 and p53 activity started as a result apoptosis takes place.
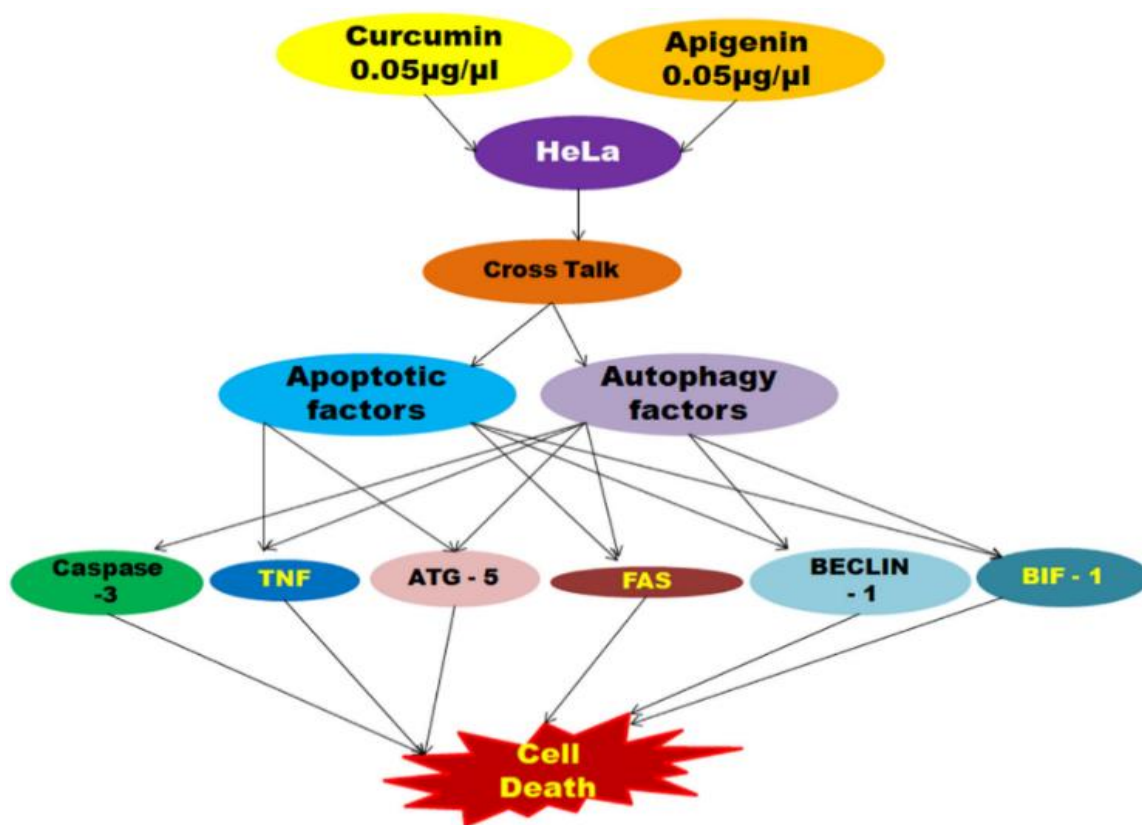
Figure 3: In cervical cancer cells HeLa showed cross-talk molecular cell death apoptosis and autophagy after treatment of Curcumin and Apigenin in a combined way. Both apoptotic factors (caspase, TNF, and ATG – 5) as well as autophagy factors (FAS, BECLIN – 1and BIF – 1) enhance both types of cell death [45].
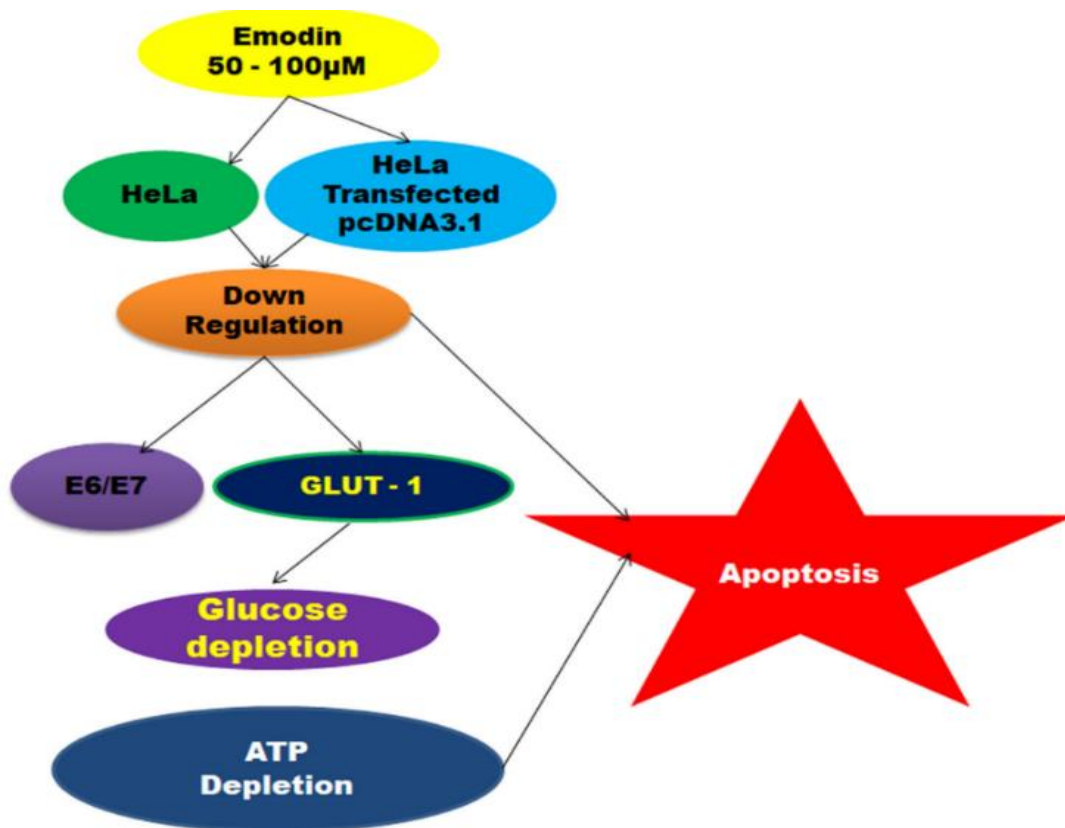

Figure 4a: Emodin promotes apoptosis in HeLa and transfected HeLa (transfected by pcDNA3.1). Which down regulates E6/E7 oncogene and Glucose depletion inhibits ATP formation. ATP level would be depleted as a result of cell death occurs.
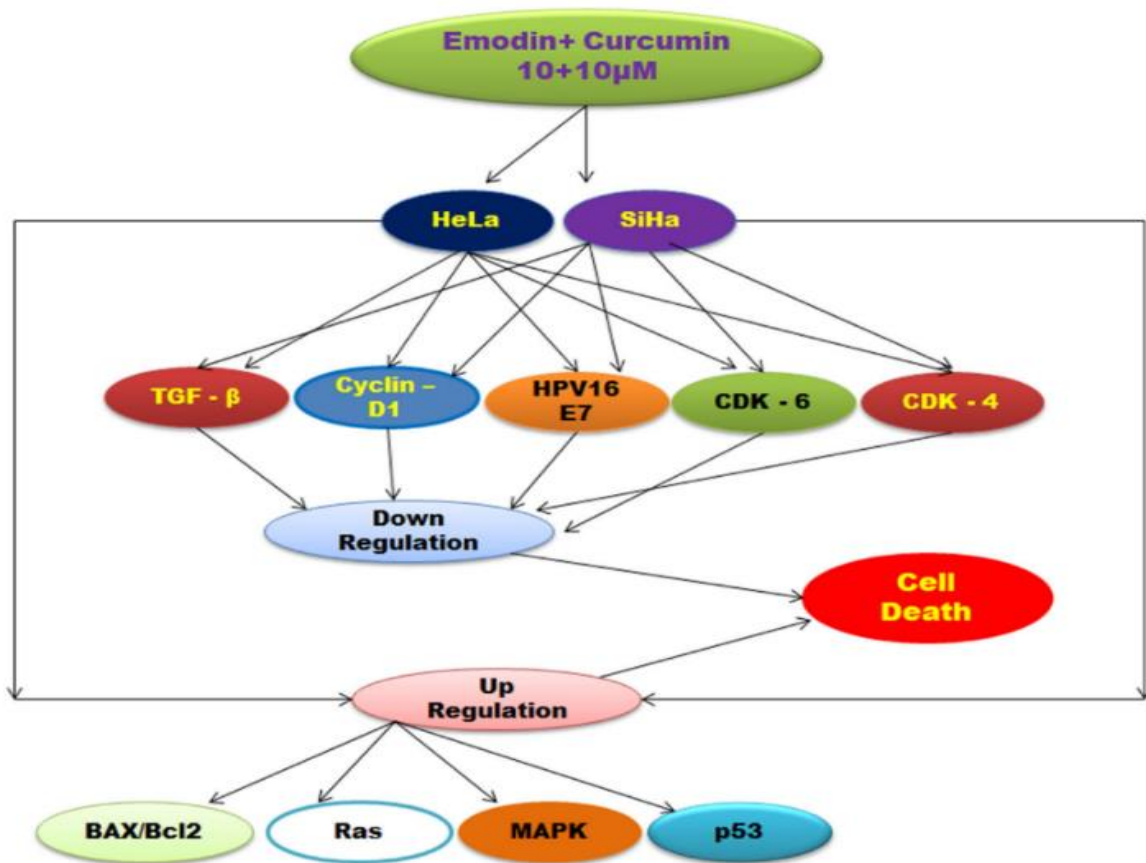
Figure 4b: Both Curcumin and Emodin mediate HeLa and SiHa cell death by downregulation of some anti-apoptotic factors (TGF – β, Cyclin – D, CDK – 6, CDK – 4) and switch on some apoptotic factors (Ras, MAPK, p53) leads to apoptotic death of both HeLa and SiHa [53].
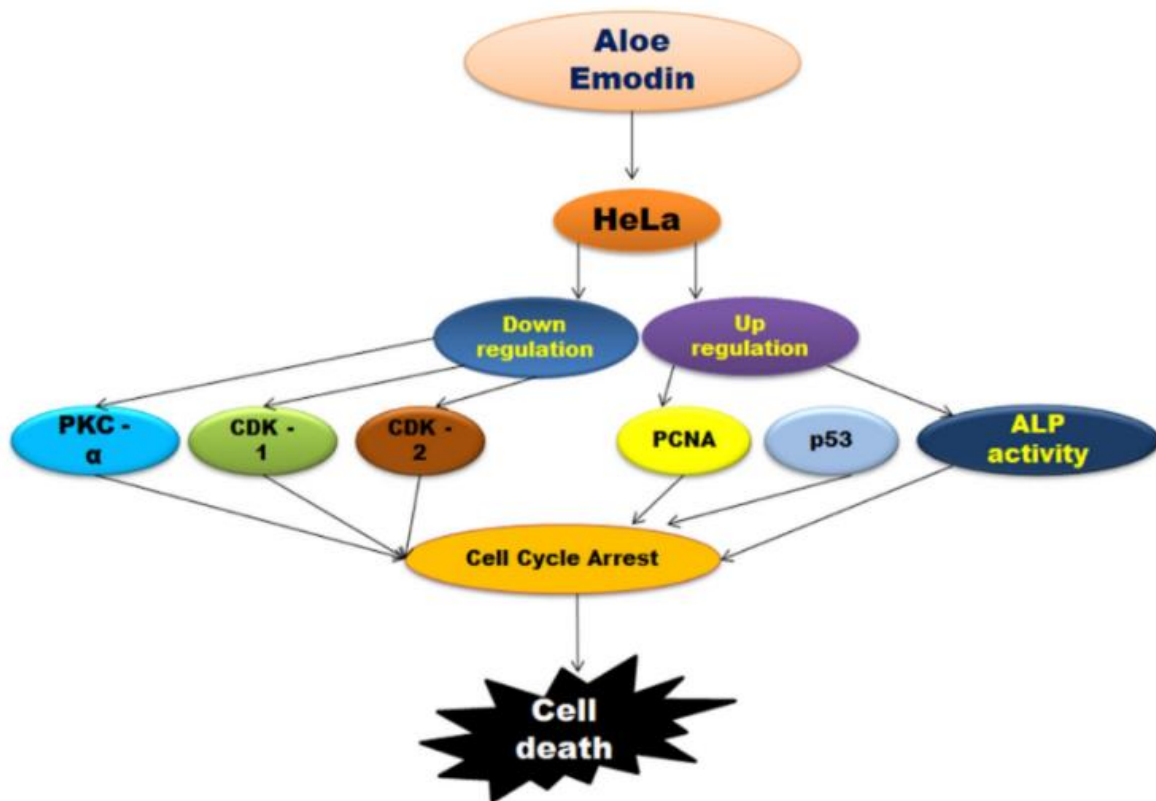
Figure 5: Aloe Emodin shows HeLa cell death by upregulation of PCNA, p53, and ALP activity and down-regulates PKC – α, CDK – 1, and CDK – 2 which leads to cell cycle arrest [49].
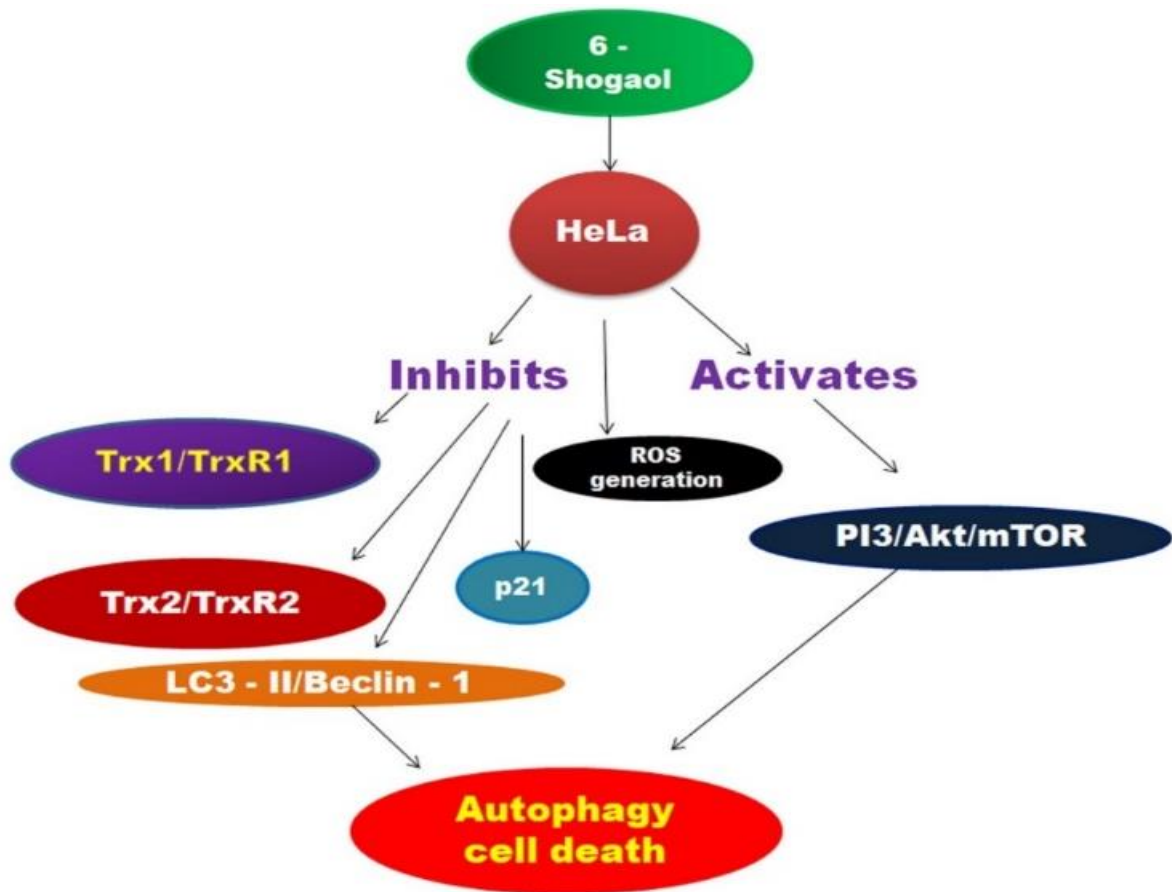
Figure 6: ROS generation takes place after treatment of 6 – shogaol treatment consequently PI3/Akt/mTOR activation leads to HeLa cell death (autophagy) shown in the schematic flow diagram [63].
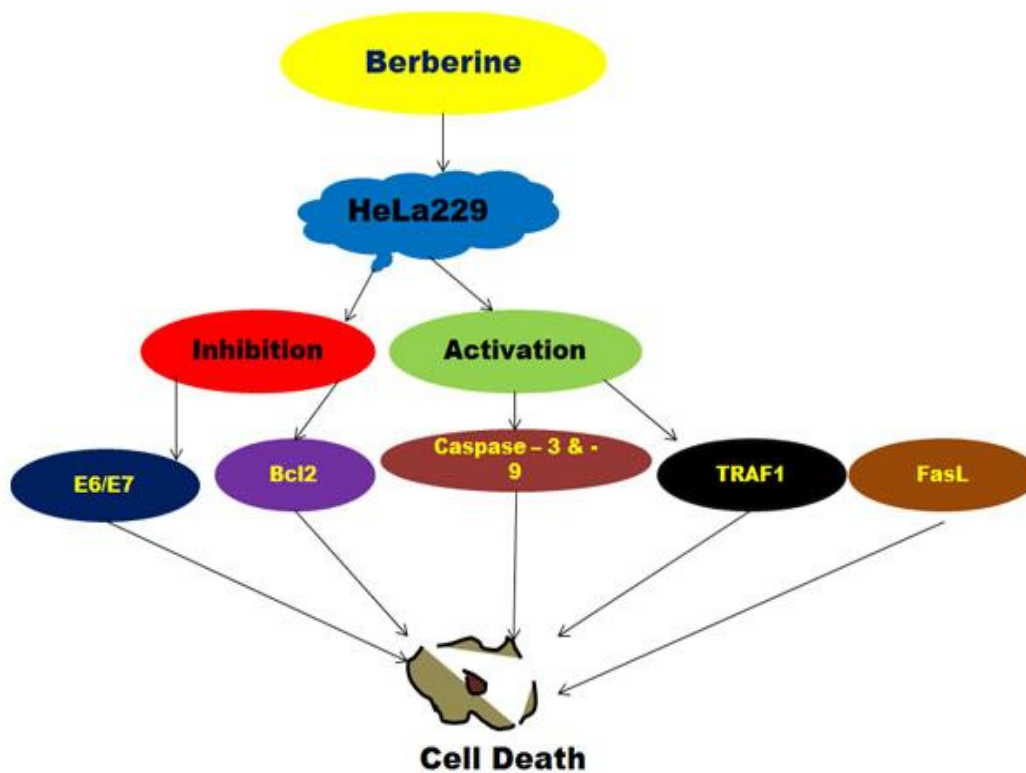

Figure 7: Onco protein E6 and E7 blocked by Berberine in HeLa229 cells. On the other hand, TRAF – 1 as well as FasL activation leads to apoptotic death of cervical cancer cell HeLa229.
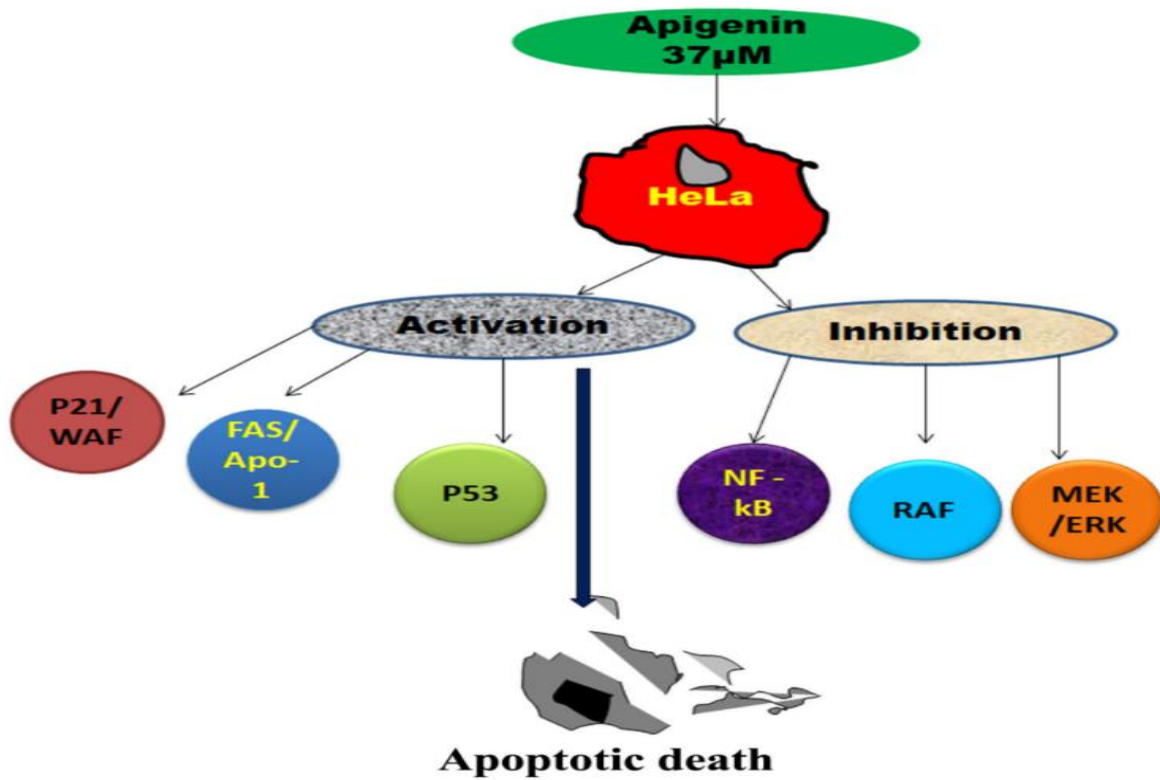
Figure 8:Proposed mechanism of cervical cancer cell (HeLa) death after treatment of Apigenin compound. It is shown that Apigenin activates p21/WAF, FAS/Apo – 1, and p53 genes, whereas NF – kB, RAF and MEK/ERK down regulation occurred.
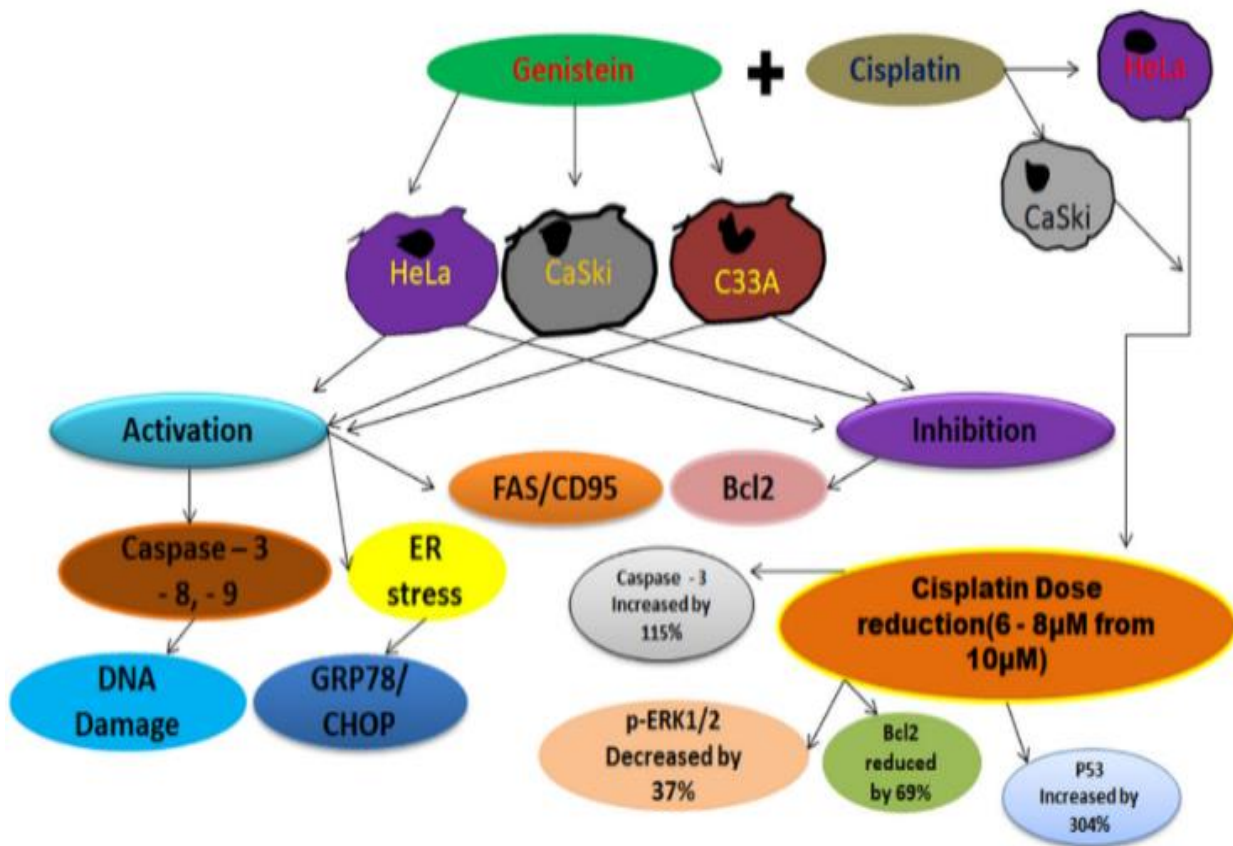
Figure 9: Genistein and Cisplatin (a conventional anti-cancer drug) induced three cervical cancer cell death (HeLa, CaSki and C33A). Genistein reduces the dose of Cisplatin and enhances cell death via Caspase -3, -8, - 9 as well as ER stress response way. Even the dose of Cisplatin was reduced by 6 – 8 µM from 10µM.

Table 1: Some important phytochemicals and their role in cervical cancer cell death are summarized. The functions and mechanism of Cell death of the above phytochemicals are discussed in the main manuscript.

Name of the compound	Source plant	Cervical cancer cell line	Major signalling pathway	References
Andrographolide labdane diterpenoid	*Andrographis paniculata* Root	HeLa	P[53] mediated path way	[22]
Curcumin biphenolic compound	*Curcuma longa*	HeLa,SiHa, CasKi	Down-regulation of Bcl – 2, Bcl – xl and activation of the caspase pathway	[32],[37]
Emodin is trihydroxyanthraquinone	*Rumex abyssinicus, Polygonum cuspidatum, Reynoutria japonica*	HeLa, SiHa	Inhibition of oncogene E6/E7, glucose depletion,ATP depletion, Wnt/β – Catenin signalling	[51]
Aloe-emodin(anthraquinone)	*Rhamnus frangula, Rheum palmatum* and *Aloe barbadensis*	HeLa	P53 pathway, ALP activity, cell cycle arrest	[49]
6-Shogaol	*Zingiber officinale*	HeLa	PI3K/Akt/mTOR signalling pathway and ROS. Autophagy death	[61]
Berberine an alkaloid compound	*Hydrastis Canadensis, Berberis vulgaris,* and *Coptis chinensis*	HeLa229	E6/E7, Bcl2 inhibition, Caspase activation	[71]
Apigenin is a flavonoid compound	*Hypericum perforatum, Matricaria chamomilla,*	HeLa	upregulation of the p21/WAF pathway, p53 activation	[79]
Genistein an isoflavone	*Genista tinctoria*	HeLa and CasKi	Bcl2 inhibition, caspase activation, FAS/CD95 activation, DNA damage	[89]
Allicin	*Allium sativum*	SiHa, Caski	Inhibiting NRF2 expression, p21WAF1/CIP1 and p27KIP1 inhibition.	[96]
Saikosaponin, a triterpenoid glycoside compound	*Bupleurum falcatum*		Down-regulation PI3K/Akt signalling, Bax is upregulated	[104]
Eugenol(4-allyl-2-methoxyphenol) combined with Cisplatin and X - ray	*Syzygium aromaticum*	HeLa	E –cadherin expression	[117]
Fisetin is a flavonoid compound	Strawberry, Apple	HeLa	caspase–3 and caspase–8 activation mediated by ERK1/2	[123]
Oleanolic acid: a pentacyclic triterpenoid compound	Olive, Garlic	HeLa	GPX4 and GSH drop-down and ferroptosis happened	[131]

4. Conclusions

The overall study about phytochemicals for the treatment of cervical cancer has expressed that a more extensive study of cell death is required in vivo and in vitro models. To overcome cervical cancer-related problems we have to search for new weapons to treat the disease. Therapeutic improvement is required to combat such type of deadliest cancer. Phytochemicals are efficient agents that can be used to overcome the hazardous effects of conventional synthetic drugs which are used for cervical cancer treatment. The main problems in cervical cancer treatment are drug resistance, side effects survival rate after treatment, and even the high cost of treatment. Conventional radiation therapy is applied but in high doses effects on the patient have fatal effects. A dozen of phytochemicals are now available to treat cervical cancer. Some phytochemicals like resveratrol, terpenoid compounds, di-terpenoids, and Polyphenolic compounds are very effective for apoptotic and autophagy cell death of malignant cells. They are controlled by different molecular pathways like caspase-dependent pathways, mitochondrial pathways, intrinsic pathways, extrinsic pathways, etc. Even some phytochemicals act combined way on cervical malignant cells. The synergistic effect of drugs can be chosen for better results for such deadliest type of disease. Some phytochemicals enhance molecular cell death of malignant cells by altering radio resistance properties. Hence, many researchers have reported that essential phytochemicals enhance radio sensitization of conventional radiotherapy techniques to combat cervical cancer. Several molecular functions are switched on, which have been studied in vitro and in vivo methods. So, it can be concluded that phytochemicals have multi-dimensional action against cancer cell proliferation. In this context, proper molecular signaling pathway detection is an important task that demands more regroup studies.

References

[1]   J. S. Butel. (2000). Viral carcinogenesis: revelation of molecular mechanisms and etiology of human disease. Carcinogenesis. 21 (3): 405–426.

[2]   M. E. McLaughlin-Drubin, K. Munger. (2008). Viruses Associated with Human Cancer. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 1782 (3): 127–150.

[3]   D. Martin, J. S. Gutkind. (2009). Human tumor-associated viruses and new insights into the molecular mechanisms of cancer. Oncogene. 27: S31–S42.

[4]   M. Aarthy, U. Panwar, S. K. Singh. (2020). Structural dynamic studies on identification of EGCG analogs for the inhibition of Human Papillomavirus E7. Scientific Reports. 10 (1): 8661.

[5]   L. S. Massad. (2018) Replacing the Pap Test with Screening Based on Human Papillomavirus Assays. Journal of American Medical Association. 320 (1): 35-37.

[6]   H. Zhu, H. Luo, W. Zhang, Z. Shen, X. Hu, X. Zhu. (2016). Molecular mechanisms of cisplatin resistance in cervical cancer. Drug design, development and therapy. 1885-1895.

[7]   M. Sohel, H. Sultana, T. Sultana, M. Al Amin, S. Akhtar, M. C. Ali, Z. B. Rahim, M. A. Hossain, A. Al Mamun, M. N. Amin, R. Dash. (2022). Chemotherapeutic potential of hesperetin for cancer treatment, with mechanistic insights: a comprehensive review. Heliyon. 8: (1).

[8]   M. A. Moga, A. Balan, C. V. Anastasiu, O. G. Dimienescu, C. D. Neculoiu, C. Gavris. (2018). An Overview on the Anticancer Activity of Azadirachta indica (Neem) in Gynecological Cancers. International Journal of Molecular Science. 19 (12): 3898.

[9]   R. H. Liu. (2004). Potential synergy of phytochemicals in cancer prevention: mechanism of action. Journal of Nutrition. 134 (12): 3479S-3485S.

[10]   S. L. Chen, H. Yu, H. M. Luo, Q. Wu, C. F. Li, A. Steinmetz. (2016). Conservation and sustainable use of medicinal plants: problems, progress, and prospects. Chinese Medicine. 11: 1-10.

[11]   J. Burns, T. Yokota, H. Ashihara, M. E. Lean, A. Crozier. (2002). Plant foods and herbal sources of resveratrol. Journal of Agriculture Food Chemistry. 50 (11): 3337-3340.

[12]   D. Choudhury, A. Das, A. Bhattacharya, G. Chakrabarti. (2010) Aqueous extract of ginger shows anti-proliferative activity through disruption of microtubule network of cancer cells. Food Chemistry and Toxicology. 48 (10): 2872-2880.

[13]   Y. Li, C. Xu. (2017). Human papillomavirus-related cancers. Infectious agents associated cancers: Epidemiology and molecular biology. 23-34.

[14]   F. Bray, J. Ferlay, I. Soerjomataram, R. L. Siegel, L. A. Torre, A. Jemal. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 68 (6): 394-424.

[15]   K. Raju. (2015). Virus and cervical cancer: role and implication: a review. Biomedical Research and Therapy. 2: 1-11.

[16]   P. Basu, D. Banerjee, P. Singh, C. Bhattacharya, J. Biswas. (2013). Efficacy and safety of human papillomavirus vaccine for primary prevention of cervical cancer: A review of evidence from phase III trials and national programs. South Asian journal of cancer. 2 (04): 187-192.

[17]   L. Galluzzi, I. Vitale, J. M. Abrams, E. S. Alnemri, E. H. Baehrecke, M. V. Blagosklonny, T. M. Dawson, V. L. Dawson, W. S. El-Deiry, S. J. C. D. Fulda, E. Gottlieb. (2012). Molecular definitions of cell death subroutines: recommendations of the Nomenclature Committee on Cell Death 2012. Cell Death & Differentiation. 19 (1): 107-120.

[18]   J. F. Kerr, A. H. Wyllie, A. R. Currie. (1972). Apoptosis: a basic biological phenomenon with wide ranging implications in tissue kinetics. British journal of cancer. 26 (4): 239-257.

[19]   S. Elmore. (2007). Apoptosis: A Review of Programmed Cell Death. Toxicology and Pathology. 35 (4): 495–516.

[20]   C. J. Kim, S. J. Um, T. Y. Kim, E. J. Kim, T. C. Park, S. J. Kim, S. E. Namkoong, J. S. Park. (2000). Regulation of cell growth and HPV genes by exogenous estrogen in cervical cancer cells. International Journal of Gynecological Cancer. 10 (2): 157-164.

[21] A. Derakhshan, Z. Chen, C. V. Waes. (2017). Therapeutic Small Molecules Target Inhibitor of Apoptosis Proteins in Cancers with Deregulation of Extrinsic and Intrinsic Cell Death Pathways. Clinical Cancer Research. 23 (6): 1379–1387.

[22] J. Zhou, G. D. Lu, C. S. Ong, C. N. Ong, H. M. Shen. (2008). Andrographolide sensitizes cancer cells to TRAIL-induced apoptosis via p53-mediated death receptor up-regulation. Molecular Cancer Therapeutics. 7 (7): 2170 – 2180.

[23] J. A. Munguía-Moreno, J. Diaz-Chavez, E. García-Villa, M. E. Albino-Sanchez, D. Mendoza-Villanueva, R. Ocadiz-Delgado, J. Bonilla-Delgado, A. Marín-Flores, E. M. Cortés-Malagón, E. Alvarez-Rios, A. Hidalgo-Miranda. (2018). Early synergistic interactions between the HPV16-E7 oncoprotein and 17β-oestradiol for repressing the expression of Granzyme B in a cervical cancer model. International journal of oncology. 53 (2): 579-591.

[24] M. A. Hall, J. L. Cleveland. (2007). Clearing the TRAIL for Cancer Therapy. Cancer Cell. 12 (1): 4-6.

[25] S. Wang, W. S. El-Deiry. (2003). TRAIL and apoptosis induction by TNF-family death receptors. Oncogene. 22 (53): 8628–8633.

[26] H. N. LeBlan, A. Ashkenazi. (2003). Apo2L/TRAIL and its death and decoy receptors. Cell Death & Differentiation. 10 (1): 66-75.

[27] S. Shankar, R. K. Srivastava. (2004). Enhancement of therapeutic potential of TRAIL by cancer chemotherapy and irradiation: mechanisms and clinical implications. Drug Resistance Updates. 7 (2): 139–156.

[28] M. S. Ricci, S. H. Kim, K. Ogi, J. P. Plastaras, J. Ling, W. Wang. (2007). Reduction of TRAIL-Induced Mcl-1 and cIAP2 by c-Myc or Sorafenib Sensitizes Resistant Human Cancer Cells to TRAIL-Induced Death. Cancer Cell. 12 (1): 66–80.

[29] C. Palacios, R. Yerbes, A. Lopez-Rivas. (2006). Flavopiridol induces cellular FLICE-inhibitory protein degradation by the proteasome and promotes TRAIL-induced early signaling and apoptosis in breast tumor cells. Cancer Research. 66 (17): 8858–8869.

[30] C. Niederau, E. Göpfert. (1999). The effect of chelidonium- and turmeric root extract on upper abdominal pain due to functional disorders of the biliary system. Results from a placebo-controlled double-blind study. Medizinische Klin (Munich). 94 (8): 425-430.

[31] S. F. Nabavi, R. Thiagarajan, L. Rastrelli, M. Daglia, E. Sobarzo-Sanchez, H. Alinezhad, S. M. Nabavi. (2015). Curcumin: a natural product for diabetes and its complications. Current topics in medicinal chemistry. 15 (23): 2445-2455.

[32] J. Zhao, Y. Zhao. (2004). Effects of curcumin on proliferation and apoptosis of human cervical carcinoma HeLa cells in vitro. Chinese Journal of Cancer Research. 16: 225-228.

[33] G. S. Sidhu, H. Mani, J. P. Gaddipati, A. K. Singh, P. Seth, K. K. Banaudha, G. K. Patnaik, R. K. Maheshwari. (1999). Curcumin enhances wound healing in streptozotocin induced diabetic rats and genetically diabetic mice. Wound repair and regeneration. 7 (5): 362-374.

[34] H. Inano, M. Onoda, N. Inafuku, M. Kubota, Y. Kamada, T. Osawa, H. Kobayashi, K. Wakabayashi. (2000). Potent preventive action of curcumin on radiation-induced initiation of mammary tumorigenesis in rats. Carcinogenesis. 21 (10): 1835-1841.

[35] E. Yang, S. J. Korsmeyer. (1996). Molecular thanatopsis: a discourse on the bcl-2 family and cell death. Blood. 88 (2): 386 - 401.

[36] Y. Shi. (2004). Caspase activation, inhibition, and reactivation: A mechanistic view. Protein Science. 13 (8): 1979–1987.

[37] M. Singh, N. Singh. (2011). Curcumin counteracts the proliferative effect of estradiol and induces apoptosis in cervical cancer cells. Molecular Cell Biochemistry. 347: 1-11.

[38] N. M. Yunos, P. Beale, J. Q. Yu, F. Huq. (2011). Synergism from sequenced combinations of Curcumin and epigallocatechin-3-gallate with cisplatin in the killing of human ovarian cancer cells. Anticancer Research. 31 (4): 1131–1140.

[39] J. M. Arbeit, P. M. Howley, D. Hanahan. (1996). Chronic estrogen induced cervical and vaginal squamous carcinogenesis in human papillomavirus type 16 transgenic mice. Proceedings of the National Academy of Sciences of the United States of America. 93 (7): 2930–2935.

[40] M. U. Jaio, X. L. Wei. (2002). Telomere and telomerase in oncology. Cell Research.12 (1):1–7.

[41] M. Singh, N. Singh. (2009). Molecular mechanism of curcumin-induced cytotoxicity in human cervical carcinoma cells. Molecular Cell Biochemistry. 325: 107–119.

[42] Y. J. Guo, W. W. Pan, S. B. Liu, Z. F. Shen, Y. Xu, L. L. Hu. (2020). ERK/MAPK signaling pathway and tumorigenesis. Experimental and therapeutic medicine. 19 (3): 1997-2007.

[43] T. Wang, X. Wu, M. Al Rudaisat, Y. Song, H. Cheng. (2020). Curcumin induces G2/M arrest and triggers autophagy, ROS generation, and cell senescence in cervical cancer cells. Journal of Cancer. 11 (22): 6704-6715.

[44] S. Kayacan, K. Yilancioglu, A. S. Akdemir, F. K. Dagistanli, G. Melikoglu, M. Ozturk. (2018). The Effects of Apigenin and Curcumin on Autophagy Related Cell Death and Apoptosis. In Proceedings. 2 (25): 1586.

[45] Y. Takahashi, N. Tsotakos, Y. Liu, M. M. Young, J. Serfass, Z. Tang, T. Abraham, H. G. Wang. (2016). The Bif-1-Dynamin 2 membrane fission machinery regulates Atg9-containing vesicle generation at the Rab11-positive reservoirs. Oncotarget. 7 (15): 20855–20868.

[46] C. Gordy, Y. W. He. (2012). The crosstalk between autophagy and apoptosis: Where does this lead? Protein Cell. 3 (1): 17–27.

[47] S. Singh, B. Sharma, S. S. Kanwar, A. Kumar. (2016). Lead Phytochemicals for Anticancer Drug Development. Front. Plant Science. 7: 224341.

[48] D. Kumara, S. Basua, L. Parijab, D. Routa, S. Mannaa, J. Dandapat, P. R. Debata. (2016). Curcumin and Ellagic acid synergistically induce ROS generation, DNA damage, p53 accumulation and apoptosis in HeLa cervical carcinoma cells. Biomedicine & pharmacotherapy. 81: 31-37.

[49] W. Trybus, T. Kró, E. Trybus, A. Stachurska, A. Kopacz-Bednarska, G. Król. (2018). Induction of Mitotic Catastrophe in Human Cervical Cancer Cells After Administration of Aloe-emodin. Anti-cancer Research. 38 (4): 2037-2044.

[50] S. Z. Lin, W. T. Wei, H. Chen, K. J. Chen, H. F. Tong, Z. H. Wang, Z. L. Ni, H. B. Liu, H. C. Guo, D. L. Liu. (2012). Antitumor Activity of Emodin against Pancreatic Cancer Depends on Its Dual Role: Promotion of Apoptosis and Suppression of Angiogenesis. Plos one. 7 (8): e42146.

[51] P. C. Thacker, D. Karunagaran. (2015). Curcumin and Emodin Down-Regulate TGF-β Signaling Pathway in Human Cervical Cancer. Plos One. 10 (3): e0120045.

[52] J. Massague, Y. G. Chen. (2000). Controlling TGF-beta signaling. Genes Development. 14 (6): 627–644.

[53] C. Perez-Plasencia, A. Duenas-Gonzalez, B. Alatorre-Tavera. (2008). Second hit in cervical carcinogenesis process: involvement of wnt/beta-catenin pathway. International archives of Medicine. 1 (1): 10.

[54] R. Gao, X. Wu, Z. Huang, B. Wang, F. Li, H. Xu, L. Ran. (2019). Anti-tumor effect of aloe-emodin on cervical cancer cells was associated with human papillomavirus E6/E7 and glucose metabolism. Oncological Targets and Therapy. 12: 3713–3721.

[55] T. H. Chiu, W. W. Lai, T. C. Hsia, J. S. Yang, T. Y. Lai, P. P. Wu, C. Y. Ma, C. C. Yeh, C. C. Ho, H. F. Lu, W. G. Wood. (2009). Aloe-emodin induces cell death through S-phase arrest and caspase-dependent pathways in human tongue squamous cancer SCC-4 Cells. Anticancer Research. 29 (11): 4503-4511.

[56] N. Kovacevic, A. Subotic, S. Budimir, D. Grubišic. (2000). Comparative study of anthraquinones from embryogenic callus tissue and zygotic embryos of Frangula alnus and Rhamnus catharticus. Pharmaceutical Biology. 38 (5): 321-325.

[57] J. G. Lin, G. W. Chen, T. M. Li, S. T. Chouh, T. W. Tan, J. G. Chung. (2006). Aloe-emodin induces apoptosis in T24 human bladder cancer cells through the p53 dependent apoptotic pathway. The Journal of urology. 175 (1): 343-347.

[58] S. Y. Lin, J. H. Yang, T. C. Hsia, J. H. Lee, T. H. Chiu, Y. H. Wei, J. G. Chung. (2005). Effect of inhibition of aloe-emodin on N-acetyltransferase activity and gene expression in human malignant melanoma cells (A375.S2). Melanoma Research. 15 (6): 489–494.

[59] R. C. Lantz, G. J. Chen, M. Sarihan, A. M. Solyom, S. D. Jolad, B. N. Timmermann. (2007). The effect of extracts from ginger rhizome on inflammatory mediator production. Phytomedicine. 14 (2-3): 123-128.

[60] B. H. Ali, G. Blunden, M. O. Tanira, A. Nemmar. (2008). Some phytochemical, pharmacological and toxicological properties of ginger (Zingiber officinale Roscoe): a review of recent research. Food Chemistry Toxicology. 46 (2): 409–420.

[61] S. Peng, S. Yu, J. Zhang, J. Zhang. (2023). 6-Shogaol as a Novel Thioredoxin Reductase Inhibitor Induces Oxidative-Stress-Mediated Apoptosis in HeLa Cells. International Journal of Molecular Science. 24 (5): 4966.

[62] J. Zhang, D. Duan, A. Osama, J. Fang. (2021). Natural molecules targeting the Thioredoxin system and their therapeutic potential. Antioxidants & Redox Signaling. 34 (14): 1083-1107.

[63] E. Chupakhin, M. Krasavin. (2021). Thioredoxin reductase inhibitors: Updated patent review (2017-present). Expert Opinion on Therapeutic Patent. 31 (8): 745-758.

[64] X. D. Pei, Z. L. He, H. L. Yao, J. S. Xiao, L. Li, J. Z. Gu, P. Z. Shi, J. H. Wang, L. H. Jiang. (2020). 6-Shogaol from ginger shows anti-tumor effect in cervical carcinoma via PI3K/Akt/mTOR pathway. European Journal of Nutrition. 60 (5): 1-13.

[65] R. Hu, P. Zhou, Y. B. Peng, X. Xu, J. Ma, Q. Liu, L. Zhang, X. D. Wen, L. W. Qi, N. Gao, P. Li. (2012). 6-Shogaol Induces Apoptosis in Human Hepatocellular Carcinoma Cells and Exhibits Anti-Tumor Activity in Vivo through Endoplasmic Reticulum Stress. PloS one. 7 (6): e39664.

[66] M. J. Youn, H. S. So, H. J. Cho, H. J. Kim, Y. Kim, J. H. Lee, J. S. Sohn, Y. K. Kim, S. Y. Chung, R. Park. (2008). Berberine, a natural product, combined with enhanced apoptosis through a mitochondria/ caspase-mediated pathway in HeLa cells. Biological and Pharmaceutical Bulletin. 31 (5): 789-795.

[67] F. Chang, L. S. Steelman, J. T. Lee, J. G. Shelton, P. M. Navolanic, W. L. Blalock, R. A. Franklin, J. A. McCubrey. (2003). Signal transduction mediated by the Ras/Raf/MEK/ERK pathway from cytokine receptors to transcription factors: potential targeting for therapeutic intervention. Leukemia (08876924). 17 (7).

[68] H. Talarmin, C. Rescan, S. Carious, D. Glaise, G. Zanninelli, M. Bilodeau, P. Loyer, C. Guguen-Guillouzo, G. Baffet. (1999). The mitogen-activated protein kinase kinase/extracellular signal-regulated kinase cascade activation is a key signaling pathway involved in the regulation of G1 phase progression in proliferating hepatocytes. Molecular and cellular biology. 19 (9): 6003-6011.

[69] Y. T. Ho, J. S. Yang, T. C. Li, J. J. Lin, J. G. Lin, K. C. Lai, C. Y. Ma, W. G. Wood, J. G. Chung. (2009). Berberine suppresses in vitro migration and invasion of human SCC-4 tongue squamous cancer cells through the inhibitions of FAK, IKK, NF-κB, u-PA and MMP-2 and-9. Cancer letters. 279 (2): 155-162.

[70] B. Lu, M. Hu, K. Liu, J. Peng. (2010). Cytotoxicity of berberine on human cervical carcinoma HeLa cells through mitochondria, death receptor, and MAPK pathways, and in-silico drug-target prediction. In vitro Toxicology. 24 (6): 1482–1490.

[71] H. Y. Wang, H. Z. Yu, S. M. Huang, Y. L. Zheng. (2016). p53, Bcl-2 and cox-2 are involved in berberine hydrochloride-induced apoptosis of

HeLa229 cells. Molecular Medicine Report. 14 (4): 3855-3861.

[72] S. Mahata, A. C. Bharti, S. Shukla, A. Tyagi, S. A. Husain, B. C. Das. (2011). Berberine modulates AP-1 activity to suppress HPV transcription and downstream signaling to induce growth arrest and apoptosis in cervical cancer cells. Molecular Cancer. 10: 39.

[73] Q. Zhang, Y. Sun, Y. Huang, J. Sun, C. Zhao, Y. Song, J. Wu. (2019). Combination of Berberine and matrine induces an intrinsic Apoptotic pathway and g1 cell cycle arrest in human cervical cancer cells. Acta Poloniae Pharmaceutica-Drug Research. 76 (6): 1089-1097.

[74] J. Yang, J. Fa, B. Li. (2017). Apigenin exerts anticancer effects on human cervical cancer cells via induction of apoptosis and regulation of Raf/MEK/ERK signaling pathway. Tropical Journal of Pharmaceutical Research. 17 (8): 1615-1619.

[75] H. S. Seo, M. A. Sikder, H. J. Lee, J. Ryu, C. J. Lee. (2014). Apigenin Inhibits Tumor Necrosis Factor-a-Induced Production and Gene Expression of Mucin through Regulating Nuclear Factor-Kappa B Signaling Pathway in Airway Epithelial Cells. Biomolecular Therapeutics. 22 (6): 525-531.

[76] Y. Maeda, H. Takahashi, N. Nakai, T. Yanagita, N. Ando, T. Okubo, K. Saito, K. Shiga, T. Hirokawa, M. Hara, H. Ishiguro. (2018). Apigenin induces apoptosis by suppressing Bcl-xl and Mcl-1 simultaneously via signal transducer and activator of transcription 3 signaling in colon cancer. International Journal of oncology. 52 (5): 1661-1673.

[77] R. Torkin, J. F. Lavoie, D. R. Kaplan, H. Yeger. (2005). Induction of caspase-dependent, p53-mediated apoptosis by apigenin in human neuroblastoma. Molecular Cancer Therapy. 4 (1): 1-11.

[78] D. M. Lepley, J. C. Pelling. (1997). Induction of p21/WAF1 and G1 cell-cycle arrest by the chemopreventive agent Apigenin. Molecular Carcinogenesis: Published in cooperation with the University of Texas MD Anderson Cancer Center. 19 (2): 74-82.

[79] P. W. Zheng, L. C. Chiang, C. C. Lin. (2005). Apigenin-induced apoptosis through p53-dependent pathway in human cervical carcinoma cells. Life Science. 76 (12): 1367-1379.

[80] S. Ramos. (2007). Effects of dietary flavonoids on apoptotic pathways related to cancer chemoprevention. Journal of Nutritional Biochemistry. 18 (7): 427–442.

[81] F. Ardito, M. R. Pellegrino, D. Perrone, G. Troiano, A. Cocco, L. L. Muzio. (2017). In vitro study on anti-cancer properties of genistein in tongue cancer. Onco Targets Therapy. 10: 5405–5415.

[82] S. H. Kim, S. H. Kim, S. C. Lee, Y. S. Song. (2009). Involvement of Both Extrinsic and Intrinsic Apoptotic Pathways in Apoptosis Induced by Genistein in Human Cervical Cancer Cells. Annals of the New York Academy of Science. 1171: 196–201.

[83] S. Lacour, A. Hammann, A. Wotawa, L. Corcos, E. Solary, M. T. Dimanche-Boitrel. (2001). Anticancer Agents Sensitize Tumor Cells to Tumor Necrosis Factor-related Apoptosis-inducing Ligand-mediated Caspase-8 Activation and Apoptosis. Tumor Biology. 61 (4): 1645–1651.

[84] A. Hussain, G. Harish, S. A. Prabhu, J. Mohsin, M. A. Khan, T. A. Rizvi, C. Sharma. (2012). Inhibitory effect of genistein on the invasive potential of human cervical cancer cells via modulation of matrix metalloproteinase-9 and tissue inhibitors of matrix metalloproteinase-1 expression. Cancer Epidemiology. 36 (6): e387-e393.

[85] Y. M. Yang, Y. Yang, W. W. Dai, X. M. Li, M. JQ, L. P. Tang. (2016). Genistein-induced apoptosis is mediated by endoplasmic reticulum stress in cervical cancer cells. European Review for Medicinal and Pharmacological Science. 20 (15): 3292-3296.

[86] D. Wallach, E. E. Varfolomeev, N. L. Malinin, Y. V. Goltsev, A. V. Kovalenko, M. P. Boldin. (1999). Tumor necrosis factor receptor and fast signaling mechanisms. Annual Review Immunology. 17331–17367.

[87] E. Szegezdi, S. E. Logue, A. M. Gorman, A. Samali. (2006). Mediators of endoplasmic reticulum stress-induced apoptosis. EMBO Reports. 79 (9): 880-885.

[88] M. K. Sundaram, M. Z. Ansari, A. A. Mutery, M. Ashraf, R. Nasab, S. Rai, N. Rais, A. Hussain. (2018). Genistein induces alterations of epigenetic modulatory signatures in human cervical cancer cells. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents). 18 (3): 412-421.

[89] H. Liu, G. Lee, J. I. Lee, T. G. Ahn, S. A. Kim. (2019). Effects of genistein on anti-tumor activity of cisplatin in human cervical cancer cell lines. Obstetrics and Gynecology Science. 62 (5): 322-328.

[90] K. C. Agarwal. (1996). Therapeutic actions of garlic constituents. Medicinal Research Review. 16 (1): 111–124.

[91] P. Pandey, F. Khan, N. Alshammari, A Saeed, F. Aqil, M. Saeed. (2023). Updates on the anticancer potential of garlic organosulfur compounds and their nano formulations: Plant therapeutics in cancer management. Front Pharmacology. 14: 1154034.

[92] C. Di, C. Sun, H. Li, J. Si, H. Zhang, L. Han, Q. Zhao, Y. Liu, B. Liu, G. Miao, L. Gan. (2015). Diallyl disulfide enhances carbon ion beams–induced apoptotic cell death in cervical cancer cells through regulating Tap73 /ΔNp73. Cell Cycle. 14 (23): 3725-3733.

[93] Z. V. M. Hussain, S. K. Sivanandhan. (2021). Anti proliferative and Apoptotic Induction of Allicin in Human Lung Cancer Cell Lines. Indian Journal of Pharmaceutical Education Research. 55 (2): 566-573.

[94] R. Chintapalli, M. J. J. Murray, J. T. Murray. (2016). Heat Inactivation of Garlic (Allium sativum) Extract Abrogates Growth Inhibition of HeLa Cells. Nutrition and Cancer. 68 (5): 818 –826.

[95] S. Oommen, R. J. Anto, G. Srinivas, D. Karunagaran. (2004). Allicin (from garlic) induces caspase-mediated apoptosis in cancer cells. European Journal of Pharmacology. 485 (1-3): 97-103.

[96] Q. Zhang, D. Yang. (2019). Allicin suppresses the migration and invasion in cervical cancer cells mainly by inhibiting NRF2. Experimental Therapeutic Medicine. 17 (3): 1523-1528.

[97] H. Y. Cho, K. Kim, Y. B. Kim, H. Kim, J. H. No. (2017). Expression patterns of Nrf2 and keap1 in ovarian cancer cells and their prognostic role in disease recurrence and patient survival. International Journal of Gynecology Cancer. 27: 412-419.

[98] V. Krajka-Kuźniak, J. Paluszczak, W. Baer-Dubowska. (2017). The Nrf2-ARE signaling pathway: An update on its regulation and possible role in cancer prevention and treatment. Pharmacological reports. 69 (3): 393-402.

[99] A. A. Zimta, D. Cenariu, A. Irimie, L. Magdo, S. M. Nabavi, A. G. Atanasov, I. Berindan-Neagoe. (2019). The Role of Nrf2 Activity in Cancer Development and Progression. Cancers. 11 (11): 1755.

[100] I. A. Ansari, A. Ahmad, M. A. Imran, M. Saeed, I. Ahmad. (2021). Organosulphur Compounds Induce Apoptosis and Cell Cycle Arrest in Cervical Cancer Cells via Downregulation of HPV E6 and E7 Oncogenes. Current Medicinal Chemistry Anticancer Agents. 21 (3): 393– 405.

[101] S. J. Kang, Y. J. Lee, S. G. Kang, S. Cho, W. Yoon, J. H. Lim, S. H. Min. (2017). Caspase-4 is essential for saikosaponin-induced apoptosis acting upstream of caspase-2 and γ- H2AX in colon cancer cells. Oncotarget. 8 (59): 100433–100448.

[102] Q. Liu, L. Tan, Y. Bai, H. Liang, Y. Zhao. (2002). The research of genus Bupleurum saponins has nearly 10 years. China Journal of Chinese Materia Medica. 27 (1): 7–11.

[103] J. C. Tang, F. Long, J. Zhao, J. Hang, Y. G. Ren, J. Y. Chen, B. Mu. (2019). The effects and mechanisms by which saikosaponin-d enhances the sensitivity of human non-small cell lung cancer cells to Gefitinib. Journal of Cancer. 10 (26): 6666–6672.

[104] J. Du, D. Song, T. Cao, Y. Li, J. Liu, B. Li, L. Li. (2021). Saikosaponin-A induces apoptosis of cervical cancer through mitochondria- and endoplasmic reticulum stress-dependent pathway in vitro and in vivo: involvement of PI3K/AKT signaling pathway. Cell Cycle. 20 (21): 2221-2232.

[105] Y. Wang, L. Zhao, X. Han, Y. Wang, J. Mi, C. Wang, D. Sun, Y. Fu, X. Zhao, H. Guo, Q. Wang. (2020). Saikosaponin A inhibits triple-negative breast cancer growth and metastasis through down-regulation of CXCR4. Frontiers in Oncology. 9: 1487.

[106] F. Benencia, M. C. Courreges. (2000). In Vitro and in vivo activity of eugenol on human herpesvirus. Phytotherapy Research. 14 (7): 495–500.

[107] G. Kayaoglu, H. Erten, T. Alacam, D. Orstavik. (2005). Short-term antibacterial activity of root canal sealers towards enterococcus faecalis. International endodontic journal. 38 (7): 483–488.

[108] M. Escobar-García, K. Rodríguez-Contreras, S. Ruiz-Rodríguez, M. Pierdant-Pérez, B. Cerda-Cristerna, A. Pozos-Guillén. (2016). Eugenol Toxicity in Human Dental Pulp Fibroblasts of Primary Teeth. The Journal of Clinical Pediatric Dentistry. 40 (4): 312-318.

[109] P. Tammannavar, C. Pushpalatha, S. Jain, S. V. Sowmya. (2013). An unexpected positive hypersensitive reaction to eugenol. British Medical Journal Case Report. PMC3794103.

[110] D. Slamenova, E. Horvathova, L. Wsolova, M. Sramkova, J. Navarova. (2009). Investigation of anti-oxidative, cytotoxic, DNA damaging, and DNA-protective effects of plant volatiles eugenol and borneol in human-derived HepG2, Caco-2, and VH10 cell lines. Mutation Research. 677 (1-2): 46–52.

[111] M. Fathy, M. A. Fawzy, H. Hintzsche, T. Nikaido, T. Dandekar, et. al. (2019). Eugenol Exerts Apoptotic Effects and Modulates the Sensitivity of HeLa Cells to Cisplatin and Radiation. Molecules. 24 (21): 3979.

[112] B. S. Park, Y. S. Song, S. B. Yee, B. G. Lee, S. Y. Seo, Y. C. Park, J. M. Kim, H. M. Kim, Y. H. Yoo. (2005). Phospho-ser15-p53 translocates into mitochondria and interacts with Bcl-2 and Bcl-xL in eugenol-induced apoptosis. Apoptosis. 10 (1): 193–200.

[113] J. L. Yi, S. Shi, Y. L. Shen, L. Wang, H. Y. Chen, J. Zhu, Y. Ding. (2015). Myricetin and methyl eugenol combination enhances the anticancer activity, cell cycle arrest, and apoptosis induction of cis-platin against HeLa cervical cancer cell lines. International Journal of Clinical and Experimental Pathology. 8 (2): 1116–1127.

[114] Z. Jiang, Q. Song, R. Zeng, J. Li, J. Li, X. Lin, X. Chen, J. Zhang, Y. Zheng. (2016). MicroRNA-218 inhibits EMT, migration and invasion by targeting SFMBT1 and DCUN1D1 in cervical cancer. Oncotarget. 7 (29): 45622-45636.

[115] A. Bermudez, N. Bhatla, E. Leung. (2015). Cancer of the cervix uteri. International Journal of Gynecology Obstetrics. 131: S88–95.

[116] Y. I. Petrova, L. Schecterson, B. M. Gumbiner. (2016). Roles for E-cadherin cell surface regulation in cancer. Molecular Biology of Cell. 27 (21): 3233–3244.

[117] H. K. Permatasari, A. B. Effendi, F. R. Qhabibi, F. Fawwaz, A. Dominique. (2021). Eugenol isolated from Syzygium aromaticum inhibits HeLa cancer cell migration by altering epithelial-mesenchymal transition protein regulators. Journal of Applied Pharmaceutical Science. 11 (5): 049-053.

[118] Y. J. Moon, X. Wang, M. E. Morris. (2006). Dietary flavonoids: effects on xenobiotic and carcinogen metabolism. In Vitro Toxicology. 20 (2): 187–210.

[119] K. A. Kang, M. J. Piao, J. W. Hyun. (2015). Fisetin induces apoptosis in human non-small lung cancer cells via a mitochondria-mediated pathway. In Vitro Cellular and Developmental Biology-Animal. 51 (3): 300–309.

[120] Y. C. Chen, S. C. Shen, W. R. Lee, H. Y. Lin, C. H. Ko, C. M. Shih, L. L. Yang. (2002). Wogonin and fisetin induction of apoptosis through activation of caspase 3 cascade and alternative expression of p21 protein in hepatocellular carcinoma cells SK-HEP-1. Archives of toxicology. 76: 351-359.

[121] T. W. Kim. (2023). Fisetin, an Anti-Inflammatory Agent, Overcomes Radio-resistance by Activating the PERK-ATF4-CHOP Axis in Liver Cancer. International Journal of Molecular Science. 24 (10): 9076.

[122] C. Dong, R. J. Davis, R. A. Flavell. (2002). MAP kinases in the immune response. Annual Review Immunology. 20: 55–72.

[123] T. H. Ying, S. F. Yang, S. J. Tsai, S. C. Hsieh, Y. C. Huang, D. T. Bau, Y. H., Hsieh. (2012). Fisetin induces apoptosis in human cervical cancer HeLa cells through ERK1/2-mediated activation of caspase-8-/caspase-3-dependent pathway. Archives of toxicology. 86 (2): 263–273.

[124] N. Afroze, P. Sreepoorna, J. Shafarin, K. Bajbouj, M. Hamad, M. K. Sundaram, S. Haque, A. Hussain. (2022). Fisetin Deters Cell Proliferation, Induces Apoptosis, Alleviates Oxidative Stress and Inflammation in Human Cancer Cells, HeLa. International Journal of Molecular Science. 23 (3): 1707.

[125] N. Yahfoufi, N. Alsadi, M. Jambi, C. Matar. (2018). The immunomodulatory and anti- inflammatory role of polyphenols. Nutrients. 10 (11): 1618.

[126] C. M. Bagi, D. F. Gebhard, C. J. Andresen. (2012). Antitumor effect of vascular endothelial growth factor inhibitor sunitinib in preclinical models of hepatocellular carcinoma. European Journal of Gastroenterology and Hepatology. 24 (5): 563–74.

[127] M. T. Lin, C. L. Lin, T. Y. Lin, C. W. Cheng, S. F. Yang, C. L. Lin, C. C. Wu, Y. H. Hsieh, J. P. Tsai. (2015). Synergistic effect of fisetin combined with sorafenib in human cervical cancer HeLa cells through activation of death receptor-5 mediated caspase-8/caspase-3 and the mitochondria-dependent apoptotic pathway. Tumor Biology. 37: 6987–6996.

[128] V. Ramakrishnan, M. Timm, J. L. Haug, T. K. Kimlinger, L. E. Wellik, T. E. Witzig. (2010). Sorafenib, a dual Raf kinase/vascular endothelial growth factor receptor inhibitor has significant anti-myeloma activity and synergizes with common anti-myeloma drugs. Oncogene. 29 (8): 1190–1202.

[129] S. F. Teixeira, R. A. D. Azevedo, M. A. C. Salomon, S. D. Jorge, D. Levy, S. Bydlowski, C. P. Rodrigues, C. R. Pizzo, J. A. M. Barbuto, A. K. Ferreira. (2014). Synergistic anti-tumor effects of the combination of a benzofuroxan derivate and sorafenib on NCI-H460 human large cell lung carcinoma cells. Biomedicine & Pharmacotherapy. 68 (8): 1015-1022.

[130] H. C. Pal, A. C. Diamond, L. R. Strickland, J. C. Kappes, S. K. Katiyar, C. A. Elmets, M. Athar, F. Afaq. (2015). Fisetin, a dietary flavonoid, augments the anti-invasive and antimetastatic potential of sorafenib in melanoma. Oncotarget. 7 (2): 1227-1241.

[131] Z. Chen, K. Y. Huang, Y. Ling, M. Goto, H. Q. Duan, X. H. Tong, Y. L. Liu, Y. Y. Cheng, S. L. Morris-Natschke, P. C. Yang, S. L. Yang. (2019). Discovery of an oleanolic acid/hederagenin-nitric oxide donor hybrid as an EGFR tyrosine kinase inhibitor for non-small-cell lung cancer. Journal of Natural Product. 82 (11): 3065-3073.

[132] C. Sanchez-Quesada, A. Lopez-Biedma, J. Gaforio. (2015). Oleanolic acid, a compound present in grapes and olives, protects against genotoxicity in human mammary epithelial cells. Molecules. 20 (8): 13670 – 13688.

[133] X. Sun, Z. Ou, R. Chen, X. Niu, D. Chen, R. Kang, D. Tang. (2016). Activation of the p62- Keap1-NRF2 pathway protects against ferroptosis in hepatocellular carcinoma cells. Hepatology. 63 (1): 173 – 184.

[134] H. Yuan, X. Li, X. Zhang, R. Kang, D. Tang. (2016). Identification of ACSL4 as a biomarker and contributor of ferroptosis. Biochemical and biophysical research communications. 478 (3): 1338–1343.