Review

Ellagic Acid: A potent Radio-sensitizer in Cancer Radiotherapy

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Abstract: The cytotoxic effect of radiotherapy of cancer is limited due to commonly observed radio-resistance in the clinic. It is, therefore, necessary to develop new strategies to enhance the radiosensitivity of tumor cells by combining therapeutic drugs with optimized radiation doses to improve the treatment outcome. Over the years, research has gained momentum in studying various herbal drugs that have the potential to sensitize tumor cells to ionizing radiation. Herbal drugs possess the property of non-toxic doses to higher concentrations and thereby minimize the undesirable side effects. Our lab recently reported the combined effects of radiation and ellagic acid (EA) in generation of increasing ROS as a function of radiation dose in HeLa thereby exhibiting the radiosensitizing effect. This article reviews the mechanism of action of EA involving ROS, cell cycle arrest, apoptosis and activation of signaling molecular cascade in the observed radiosensitization of some tumor cell lines and suggests its potential usefulness in clinic for improving cancer radiotherapy which uses the mechanism of increasing the oxidative stress through generation of ROS in cancer cells.

Keywords: Radiosensitiser, Cancer, Radiation therapy, Ellagic Acid

I. Introduction
Cancer therapy still depends on ionizing radiation as one of its most effective tools. Ionizing radiation works by inducing damage not only at the molecular, cellular, tissues, organ system but the whole body either by direct or indirect effects. A basic phenomenon of reactive oxygen species and reactive nitrogen species formation takes place when a biological system is exposed to radiation. These reactive species formed have a potential to damage DNA, proteins, lipids; including the plasma membrane [1]. Lately, clinicians have commonly observed occurrence of tumors either being poorly responsive or even non-responsive to therapeutic drugs and radiotherapy thereby limiting cancer treatment. Thus it becomes a prime responsibility of researchers to embark on active research in providing multimodality treatment involving radiotherapy and systemic drugs. Novel systemic treatment choices also include immunotherapy, and are no strict chemotherapeutic agents. Hence logical strategy would be to augment the tumor cell cytotoxicity but concurrently protecting normal cells from the adverse effects. This has led to remarkable awareness among researcher in identifying herbal compounds which would induce apoptosis in tumor cells but exhibit a protective effect on normal cells when used in combination with radiotherapy. The success of this strategy would pave way for improvement of cancer radiotherapy. Certain naturally occurring compounds which are a part of the human diet and are devoid of toxicity within certain doses are of major interest to be considered as a choice for radiation therapy [2]. This article aims to summarize natural products as potential radiosensitizer with an emphasis to EA.

1. Radiomodulators: Herbals

Polyphenols in fruits and vegetables exhibit cytotoxic effects in various pathological conditions including cancer. Since these polyphenolic compounds show diverse properties like anti-proliferative, prooxidative, anti-oxidative, immune simulative, antimicrobial, anti-inflammatory, etc. they are receiving more attention as radioprotectors and radiosensitizer than those currently available drugs. [3]. These natural compounds have a long history of safety in terms of traditional usage in humans for several years, their holistic mode of action and countering of the toxic effects of certain constituents,
synergism and novel source of new drugs etc. have been considered worthy as radiomodulators.

Radiosensitiser are compounds that when combined with radiation would achieve greater tumor inactivation than would have been expected from the additive effect of each modality [4]. They are also defined as agents that do not exhibit a significant therapeutic effect of their own, but act to enhance the therapeutic effect of radiation [5]. They are believed to work at different levels of cellular phases and one of the suggested mechanisms of treating cells with the particular radiosensitizer before irradiation probably consists in synchronizing them in sensitive cell cycle phase. Studies have shown presence of compound during irradiation amplifies the effects by multi-factorial mechanisms including toxic reactions of free radicals [6-11]. Natural products used as radiosensitizer when included after radiation show their effect by inhibiting repair of the radiation-induced lethal and sub-lethal damage apart from down-regulation of numerous pro-survival factors [2].

1.1. Herbals and apoptosis

Compounds to be considered as potential adjuvants in radiotherapy should indicate a strong mechanistic rationale for a differential response between tumor and normal tissues. Lethal properties of ionizing radiation can be enhanced by the use of radiation sensitizers when administered in conjugation with radiation therapy. They upsurge radiosensitivity without being inherently toxic and give rise to substantial increase in the radiation sensitivity of neoplasm over normal tissues. Much of the laboratory work which has been carried out in vitro has shown that many herbal compounds act as radiosensitisers [7-10]. Their practical approach depends on the exploitation of the difference between the tumor cells and normal cells. Hypoxic cells have been seen in many tumor cells which are resistance to lethal effects of ionizing radiation and so they prove to be a potential barrier for the success of radiotherapy. Drugs which selectively radiosensitize hypoxic cells might be exploited in radiotherapy to increase the sterilization of resistant hypoxic areas of solid tumors which have outgrown their vascular supply. If toxicities are not additive, therefore emphasis should be laid on future work in relation to tumor selection for clinical trials, drug cytotoxicity reduction and ways for enhancing radiosensitization property of the herbal.
combinations of radiosensitizers and radioprotectors might prove more effective than either individual approach. It is the need of time to find out newer, better and efficient compounds in a large amount as good scientific data on clinical trials have accumulated during the past few years. This would definitely lead in the deeper understanding and role of radiosensitizer along with its benefits in clinical radiotherapy. The need today is to unravel the specific mechanism of action of the various bioactive compounds and understand how they act in combination with radiation.

1.2. Rationale of herbal radiosensitizer

Radiosensitizing in vitro and invivo by plant polyphenols has been recently documented for their effects on tumors [12-16]. Some of the natural products that can be used as radiosensitisers are resveratrol from *Vitis vinefera*, withaferin A from *Withaferin somnifera*, taxol from *Taxus bataccca*, ginestein from soybean etc. Some whole extracts of plants like *Azadiracta indica*, *Tinospora cordifolia* etc have also shown the radiosensitizing effects. These compounds when combined with radiation treatment show an additive effect in tumor cell killing and/or inactivation thereby were augmenting the therapeutic effect of radiation treatment [4,5]. A flavone, Flavopiridol radiosensitizer malignant glioma cells [17]. Gossypol from gossypium *species results* in regression of human prostate cancer [18]. Kasten pisula showed that radiosensitization of tumor cells is due to compromised double strand break repair and not due to enhanced apoptosis [19]. Curcumin renders radiosensitising effect by inhibiting the growth of PC-3 *human prostate cancer cell line* cells lines and down-regulating the pro-survival factors which are induced by radiation[20]. EA on a similar lines has shown to enhance the radiation mediated oxidative stress and the consequent cytotoxicity in tumor cells by decreasing the antioxidant enzymes like SOD, glutathione peroxidase and catalase [6].

2. Ellagic acid (EA)
EA is a plant-derived polyphenol, possessing antioxidant, antiproliferative, and antiatherogenic activities. Plants produce EA to protect themselves from microbiological infection and pests. EA has also been said to reduce heart disease, birth defects, liver problems, and to promote wound healing. In plants, ellagic acid is present in the form of ellagitannin, which is EA bound to a glucose molecule. It is usually present in the form of hydrolysable tannins called ellagitannins - esters of glucose with hexahydroxydiphenic acid - that when hydrolyzed yield EA. It is found in raspberries, strawberries, cranberries, walnuts, pecans, pomegranates, and other plant foods. When raspberries, strawberries, and pomegranates are freeze-dried they yield the highest levels of EA. Extracts from red raspberry leaves or seeds, pomegranates, or other sources are said to contain high levels of EA and are available as dietary supplements in capsule, powder, or liquid form.

EA seems to possess some anti-cancer properties which have been seen in a variety of cells and tissues like breast, liver, lung, colon etc. EA activates various signaling pathways, including apoptosis, protection from oxidative DNA damage, or LDL-oxidation and alteration of growth factor expression, as well as through the expression of p53, NF-kB, and PPAR family responsive genes. It can act as an anti-oxidant, and has been found to cause cell death in cancer cells in the laboratory. In other laboratory studies, EA seems to reduce the effect of estrogen in promoting the growth of breast cancer cells in tissue cultures. There are also reports that it may help the liver to break down or remove some cancer-causing substances from the blood.

EA from red raspberries causes growth cycle arrest of cancer cells, thus inhibiting cell division (mitosis) and cellular proliferation. It also prevents the destruction of a cell regulatory gene by HPV oncogenes (genes responsible for cancer induction) in cervical cells, which gene is regarded as the safeguard of normal cellular division, and which when inactivated results in abnormal cell division/proliferation. Therefore, some researchers have claimed these results mean that EA can prevent or treat cancer in humans. In vitro, in vivo research has shown that EA may slow the growth of some tumors caused by certain
carcinogens. It prevents the binding of carcinogens to DNA and strengthens connective tissue, which may keep cancer cells from spreading. It has the ability to inhibit mutations within a cell's DNA. Furthermore, it is considered to be a cancer inhibitor which has the ability to cause apoptosis in cancer cells.

Dr. Daniel Nixon who is one of the prominent scientists in cancer research at the Hollings Cancer Center in the Medical University of South Carolina has examined the ability of EA to prevent colon and cervical cancers from developing. His research and findings indicate that EA

a) Cancer causing mutagens in serum can be detoxified when EA activates the detoxifying enzymes present in the liver.

b) Binding the carcinogens to DNA/abduct formation can be prevented by EA.

c) Highly destructive oxygen free radicals can be scavenged and cleared by antioxidant property of EA.

d) EA stimulates the immune system to destroy cancer cells.

e) EA induces apoptosis in cancer cells

**EA induced apoptotic radiosensitivity** Our group investigated the potential of EA as a radiosensitizer in HeLa cells. A significant increase of 2.5 fold in ROS was observed when HeLa cells were treated with increasing concentration of EA with different doses of $\gamma$-radiation (1-6 Gy). An additive effect was observed in the generation of ROS. Percentage viability of cells significantly decreased by 46% in 24 hrs in vivo. The antioxidant enzyme system was also observed to drastically affected i.e. SOD levels decreased by 62%, catalase by 52% and GSH-Px by 52% in tumors aspirated from mice subjected to the combined treatment of radiation and EA. Also, the GR levels decreased by 26%. The mitochondrial potential also reduced by 37% in the mice treated with radiation and EA in vivo. Therefore, EA can be said to be a pro oxidant invitro at concentrations at 100umol/l in HeLa cells and generation of ROS increases with increasing concentration of EA. It can...
be concluded that EA enhances radiation-induced oxidative stress and cytotoxicity in vitro in HeLa cells and in vivo in Ehrlich Ascites Carcinoma transplanted Swiss mice [6].

In HeLa cells, the magnitude of superoxide's generation was found substantially higher than in cells either treated with radiation or EA alone. As compared to control the SOD levels decreased by 30% and GPx decreased by 47%. The p53 protein which is a pro-apoptotic protein was seen to be unregulated in HeLa cells treated with both EA and radiation compared to cells treated with either EA or radiation. HeLa cells also showed elevated Caspase-3 activity after 24 hrs when treated with EA and radiation [13].

2.1. **EA and cancer cells**

In MOLT-4, a human leukemic cell line, EA synergistically with Quercitin enhanced apoptosis [14,24]. Lansky EP et al demonstrated that EA acts against in vitro against human PC-3 prostate cancer cells exhibiting anti-tumor activities [15]. Also, at Louisiana State University Researchers showed that EA exhibited selective toxicity and apoptosis induction in cell lines like in MCF-7, Hs 578T, Caco-2, DU 145 and human prostate cancer cells. The mode of action of EA was found to be linked with decreased ATP production that is vital for the survival of cancer cell [25]. Mutation defensive properties in rat esophagus against the mutagenicity of the nitrosamine N-nitrosomethylbenzylamine (NMBA) were observed by de Boer JG et al at University of Victoria, Canada [26]. EA induced cell cycle arrest at the G1 phase in 48 h and subsequently lead to apoptosis in 72 h in T24 human bladder cancer cells in vitro [27].

2.2. **EA as radiosensitizer**

One of the commonly reported mechanisms of radiosensitization is that of enhanced generation of ROS and RNS. The final cellular response is affected by the transient surge of radiation-induced surge ROS/RNS. The incidence of this is strongly attributed to the interaction of radiation with water molecules in the biological system to produce a variety of ROS and RNS. It has been estimated that about 70% of cell injury is caused by...
hydroxyl radicals who further leads to activation of pro-inflammatory factors both in vitro and in vivo, lipid peroxidation, DNA and protein oxidation etc. [28-33]. ROS generators like diospyrin and plumbagin and β-lapachone [34-36], are pro-oxidants and therefore, can induce the apoptotic pathways in tumor cells by damaging their DNA, depolarization of the mitochondria etc. Maytansinol and vinca alkaloids augment radiation effect by interfering with the microtubules and thereby inhibiting tumor proliferation [37]. Similarly flavonoids like that of ellagic acid and silibinin are known exhibit the pro-oxidant activity and act as an inducer of intracellular oxidative stress in tumor cells which also induces the formation of reactive oxygen species of about 2.5 and 2 fold in vitro and invivo respectively. Cell viability was significantly greater in cells treated with EA prior to radiation. Tumor transplanted mice exhibited about 45% increases in splenic lymphocytes and oxidative stress persisted up to 24h.There was decreased observed in the antioxidant enzymes like superoxide dismutase, catalase, glutathione reductase in tumor cells in vivo. The transmembrane mitochondrial potential drop exhibited by EA signifies the contribution of mitochondrial permeability alterations in ROS in cells subjected to the combinatorial treatment of radiation and EA [6].

In a study of yeast rad 52 mutants, which lack recombinational DNA repair pathway, it was found that protection was solely brought about by reducing DNA damage rather than by interfering with DNA repair when EA was used in various concentrations of 100,200, and 500mM suggesting the radioprotective effect of EA [16].

In a recent study with swiss albino mice 6 Gy of Electron Beam Radiation and then treated with EA for 15 consecutive days. Test groups revealed advancement in the levels of antioxidants and antioxidative enzymes compared to irradiated group. There was a substantial reduction in the levels of membrane lipid peroxidation in the treated groups compared to irradiated control. The findings suggest the protective potential of EA on radiation-induced biochemical changes in mice may be due to its free radical scavenging and increased antioxidant levels [38].

Oral administration of natural antioxidants, EA (200 micro moles), curcumin (400 micro moles), and bixin (200 micro moles) per kilogram body weight lead to the induction of micronuclei and chromosomal aberrations produced by whole body exposure of r-radiation (1.5-3.0 Gy) in mice was found to be significantly inhibited. The inhibition of micronucleated polychromatic and normochromatic erythrocytes, led by these antioxidants was comparable to that of alpha-tocopherol (200 micro moles) administration. EA and curcumin were as effective as alpha-tocopherol in reducing the number of bone marrow cells with chromosomal aberrations and chromosomal fragments. Additionally, these antioxidants showed potential.
in inhibiting the DNA strand breaks produced that were formed in rat lymphocytes after irradiation. The study suggested that EA, curcumin and bixin showed protection towards radiation induced chromosomal damage [39]. Since EA enhanced radiation-induced oxidative stress and subsequent cytotoxicity in tumor cells in vitro and in vivo, it holds a good promise in clinics as a radiosensitizer.

2.4. EA and DNA damage

Results have indicated that EA provides protection against chromosome damage produced by radiation in normal cells. EA significantly inhibits chromosomal aberrations and micronuclei induction produced by whole body irradiation (1.5-3.0 Gy) in mice when orally administered (200 micro moles) per kilogram body weight. It induced inhibition of micronucleated polychromatic and non-mochromatic erythrocytes. EA worked as effectively as alpha-tocopherol in significantly reducing the number of bone marrow cells with chromosomal aberrations and chromosomal fragments. DNA unwinding studies in rat lymphocytes showed that when antioxidants inhibited the DNA strand breaks produced upon radiation [39].

A dose dependent study of modulation effect of EA reveals that at 10µM EA reduces the unknown adducts whereas 100uM was required to reduce the 8-oxodG [40]. It has been known that hydroxyl radical causes damages to the DNA whereas singlet oxygen plays a vital role in the generation of 8-oxo-dG [41,42]. At lower concentrations EA proves to be more effectual on hydroxyl radicals causing DNA damage. A shows elevated likeliness to bind the poly dA-dT than poly dG-dC [43,44]. This explains the discrepancy of lower dose effects. Besides, EA not only prevents the formation of DNA-carcinogen adducts by similar mechanisms [45] but also modifies the metabolism of carcinogens [46,47].

EA alters the gene expression of the LNCaP human prostate cancer cell lines which are known to be androgen sensitive altered by EA. In 48 hrs, more than twofold difference
was noted in the expression of 593 genes from untreated cells. Most interesting was the expression of alteration of p53 responsive genes and in p300, Apaf-1, NFkBp50 and PPAR families of genes involved in signaling pathways leading to growth inhibition [22].

2.5. **EA inhibits HPV oncogene expression**

Being known for it’s anti-cancer activity in vitro and in vivo; EA inhibits protein kinase CK2, and block signaling cancer related pathways that might induce tumors. Also, it’s known to restart the latent cellular defense mechanism which has been identified as a promoter of tumorogenesis. In a study of HPV 18 positive HeLa cells induced cell cycle arrest and a dose-dependent caspase dependent cell death. EA potentially inhibited the E6 and E7 viral oncogenes and the phosphorylation of CK2. p53 expression was augmented whereas a decrease in cyclin A was observed. Cytochrome C release was also seen in the cytosol and also the activation of caspase 3. Hence these results suggest the anti-tumor properties can prevent the HPV induced cervical cancer [48].

2.6. **EA associated Signaling pathways**

At $10^{-5}$ M, EA induces G1 arrest within 48 h and inhibits overall cell growth and induced apoptosis in Ca$^+$ki cells after 72 h of treatment. Activation of p21, the cdk inhibitory protein by EA suggests its role for in cell cycle regulation of cancer cells [49]. EA is one of the most interesting substances with pro-apoptotic and antioxidant action that determines apoptosis, down regulation of IGF-II, activates p21 (waf1/Cip1) a cyclin-dependent kinase inhibitor able to arrest the cell cycle at the G1, and prevents the destruction of p-53 gene by cancer cells. A multistep process inducing programmed death in cancer cells has been observed and this process inhibits the mitotic phase and blocks the cells in G1/S transition phase, prevents gene p53 destruction by cancer cells, determines IGF-II down-regulation, activates gene p21 (waf1/Cip1) and enhances NK-cell mediated antitumor immune response [25-27, 50].
EA exhibits antiangiogenic properties by specifically inhibiting VEGFR-2 and PDGFR activities and the phosphorylation of their substrates, leading to an inhibition of VEGF-induced endothelial cells migration and PDGF-induced smooth muscle cell migration, as well as to an inhibition of the morphogenic differentiation of endothelial cells into capillary-like structures [51].

At concentrations 10 to 50 mmol/L, EA stimulates apoptosis in human pancreatic adenocarcinoma cells. It decreases proliferation by up to 20-fold at 50 mmol/L. EA does not directly affect mitochondria but stimulates the mitochondrial pathway of apoptosis associated with mitochondrial depolarization, cytochrome C release, and the downstream caspase activation. NF-κB binding activity was observed to decrease dose dependently. Furthermore, inhibition of NF-κB activity using IkB wild-type plasmid prevented the effect of EA on apoptosis [52].

EA was found to be pro-oxidant in vitro at the concentration of 100 micro mol/l in HeLa cell and the generation of ROS increases with the increased concentration of EA. Tumor cells showed response to the treatment of either radiation or EA, which is implicated in its potential of increasing intracellular ROS generation, but more pronounced response was seen in cancer cells treated with the combination of radiation and EA in vitro as well as in vivo. The enhancement of radiation-induced oxidative stress by EA persisted up to 24 h [13]. Terminalia chebula a plant containing EA as a major constituent was shown to induce apoptosis in human osteosarcoma (HOS-10), breast (MCF-7) and prostate cancer cell line [50]. Apoptosis with down-regulation of insulin like growth factor (IGF-II) and activation of p21 was seen in colon cancer cells treated with EA [51]. Table 2 gives an idea of various signaling mechanisms that EA induces in different kind of tumors.

3. Conclusion and Perspectives

In view of the fact that radiotherapy fails in the later stages of cancer due to the radio-resistant tumor cells, it is most important in radiobiology to increase the oxidative damage
of the tumor cells by using a tumor-selective cytotoxic agent. Many studies have recently shown that EA exhibits the anti-proliferative and antioxidant properties in vitro as well as in vivo models because of which it has stimulated primary research into the potential health benefits of its consumption [52]. EA has a chemo protective as well as radioprotective effect in cellular models by reducing oxidative stress in normal cells [53, 54]. This oxidative stress is observed due to oxygen and several other free radical species that are associated with the induction of DNA single- and double-strand breaks. Naturally occurring antioxidants are being extensively analyzed for their ability to protect DNA against such injury in normal cells but simultaneously damage the DNA of tumor cells via ROS generation. The ameliorative effect of EA against the radiation induced biochemical alterations is attributed to its free radical scavenging properties and their ability to induce antioxidant enzymes. All these studies prove EA as an effective inducer of apoptosis and hence a potential therapeutic in the treatment of cancer cells.

Acknowledgement

We thank UGC, government of India for providing Senior Research Fellowship to Vidhula Ahire. Declaration of Interest

The authors report no conflict of interest.

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Abbreviations

RT Radiation Therapy
IR Ionizing Radiation
LET Low Energy Transfer
ROS reactive oxygen species
RNS reactive nitrogen species
EA Ellagic Acid
NF-kB nuclear Factor kappa B
PPAR peroxisome proliferator-activated receptors
HPV human papiloma virus
VEGF vascular endothelial growth factor
VEGFR vascular endothelial growth factor receptor
PDGF platelet derived growth factor
PDGFR platelet derived growth factor receptor
EC endothelial cells
SOD superoxide
GSH-Px glutathione peroxidase
GR glutathione reductase
<table>
<thead>
<tr>
<th>Herbal compound</th>
<th>Action</th>
<th>Cancer Category</th>
<th>Cell line</th>
<th>Reference</th>
</tr>
</thead>
</table>
| Curcumin        | • Inhibition of growth  
• Downregulation of prosurvival factors | Prostate cancer | LNCaP | Deeb et al. [20] |
| EA              | • Enhance radiation mediated oxidative stress  
• Decrease antioxidant enzymes | Cervical Cancer | HeLa | Bhosle et al. [6] |
| Caffeic acid    | • Deplates Glutathione  
• Inhibits NF-κB activity | Colorectal adenocarcinoma | CT26 | Chen and coworkers 2005b [7] |
| Sesamol         | • Decrease in GSH, SOD, GPx  
• Increased lipid hydroperoxides | Cervical Cancer | HeLa | Mohana et al. [57] |
| Diospyrin       | • Regulates genes of cell cycle & apoptosis  
• Downregulation of p53, p21 | Breast cancer | MCF | Kumar B. et al. [8] |
| Triphala        | • Increased tumor cell killing | Breast cancer | MCF-7; T47D | Sandhya et al. [9] |
| Tocopherol Succinate | • Decrease in cell viability  
• Increases ROS generation  
• Changes in plasma membrane fluidity | Breast cancer | MCF-7 | Kumar et al. [10] |
| Betulinic Acid  | • Increased cell cytotoxicity on radio resistant cells | Head and neck cancer | SCC9; SCC25 | Eder-Czembirek et al., 2010 [55] |
|                 | • Induce radiosensitivity in glioma cells under hypoxia | Malignant glioma | 251MG; U343MG | Bache et al., 2011 [56] |
Table 2: Mode of Action followed for EA-induced apoptosis in various cancer cell lines.

<table>
<thead>
<tr>
<th>Cancer category</th>
<th>Cell line</th>
<th>Action Mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer</td>
<td>MCF-7</td>
<td>Cycle arrest in the G0/G1 phase, γ-H2AX foci formations, drop in mitochondrial membrane potential, changes in nuclear morphology, decrease in Bcl-2, increase in Bax, PARP.</td>
<td>Ahire V. et al. [58, 59]</td>
</tr>
<tr>
<td>Cervical cancer</td>
<td>HeLa</td>
<td>Augmented oxidative stress, decrease in antioxidants enzymes like SOD, catalase, GPX; drop in MMP, increase p53, PARP, cell cycle arrest at G1 phase, induction of γ-H2AX.</td>
<td>Sushma et. al [6], Vidhula et.al [60]</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>Lncap</td>
<td>Down regulation of CYP2J2, CYP4F2 and CYPA22 mRNAs, Decreased levels of VEGF, FGF, G-CSF, HGF and IL-15</td>
<td>Luca Vanella et. al [61]</td>
</tr>
<tr>
<td>Bladder cancer</td>
<td>T24</td>
<td>Induced G0/G1-phase arrest of the cell cycle and apoptosis, increased p53 and p21, decreased CDK2, promoted caspase-3 activity</td>
<td>Li TM et. al [62]</td>
</tr>
<tr>
<td>Pancreatic adenocarcinoma</td>
<td>MIA, PaCa-2, and PANC-1</td>
<td>Decreased nuclear factor-kappa B (NF-κB) activity, loss in mitochondrial membrane potential (Δψm), cytochrome C release, and caspase-3 activation</td>
<td>Edderkaoui M. et. al [63]</td>
</tr>
<tr>
<td>Colon adenocarcinoma</td>
<td>Caco-2</td>
<td>Down-regulation bcl-XL, release of cytochrome c, caspase 9 and 3 activated, cell-cycle arrest in S phase, down-regulation of cyclins A and B1, upregulation of cyclin E, induction of apoptosis by FAS-independent, caspase 8-independent pathways.</td>
<td>Mar Larrosa et. al [64]</td>
</tr>
<tr>
<td>Ovarian carcinoma</td>
<td>ES-2 and PA-1</td>
<td>Elevates p53 and Cip1/p21, decreases cyclin D1 and E levels, induced caspase-3-mediated apoptosis by increasing the Bax/Bcl-2 ratio, arrest in G1 phase, accumulation of p53 and Cip1/p21 and reduction of cyclins D1 and E.</td>
<td>Chung YC et.al [65]</td>
</tr>
<tr>
<td>Melanoma</td>
<td>1205Lu, WM852c and A375</td>
<td>GI cell cycle arrest, increased levels of apoptosis, decreased synthesis of IL-1β and IL-8, decreased NF-κB activity.</td>
<td>Jensen JD1et. al [66]</td>
</tr>
<tr>
<td>Nasopharyngeal</td>
<td>NPC-BM1</td>
<td>Bcl-2 downregulation, DNA fragmentation, increased caspase-3 activity decreasing telomerase activity</td>
<td>Huang ST et. al [67]</td>
</tr>
<tr>
<td>carcinoma</td>
<td>increase in chromosomal DNA degradation, and hypodiploid DNA content, Significant time-dependent nuclear fragmentation, upregulating Bax and activating caspase-3. Induces apoptosis</td>
<td>Han DH et al [68]</td>
<td></td>
</tr>
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</table>
Figure 1: Commonly reported mechanisms of herbal radiosensitizer rendering radio sensitization
Figure 2: Structure of Ellagic Acid
Figure 3: Model showing effect of ionizing radiation alone and in combination with EA on cancer cell.

When the cells are treated with radiation alone there is an increase in the ROS levels. Use of chemotherapeutic dietary compound like EA (orange), drops the mitochondrial potential by release of cytochrome C (light blue), increases caspase-3 (purple) expressions pushing the cells apoptosis.
Figure 4: Some of the possible mechanisms by which EA renders radio sensitization.