Review article

Genistein Mediated Molecular Pharmacology, Cell-Specific Anti-Breast Cancer Mechanism with Synergistic Effect and in silico Safety Measurement

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Abstract

Breast cancer (BC) is the most common type of cancer in both men and women alike, but it is more prevalent in women. Natural compounds that can modulate the oncogenic process can be considered a significant anti-cancer agent for treating BC. These natural compounds are more effective than synthetic drugs, which have profound side effects on the normal cell and resistance to cancer cells. Genistein is a type of dietary phytoestrogen included in the flavonoid group with a similar structure of estrogen that might provide a strong alternative and complementary medicine to existing chemotherapeutics drugs. Several research studies demonstrated that it can target the estrogen receptor (ER), Human epidermal growth factor receptor-2 (HER2), and Breast cancer gene-1 (BRCA-1) in multiple BC cell lines, as well as sensitize cancer cell lines to this compound when used at an optimal inhibitory concentration. Genistein effectively showed anti-cancer activities through apoptosis induction, arresting cell cycle, inhibiting angiogenesis with metastasis, reducing inflammation, mammosphere formation, tumor growth, up-regulating tumor suppressor gene, and downregulating oncogene in suppressing cancer progression in vitro and animal model study. In addition, research studies have also suggested that these phytochemicals synergistically reverse the resistance mechanism of chemotherapeutic drugs, increasing the efficacy of some chemoinformatics drugs. Our review article aims to unbox and validate the molecular pharmacology in breast tissue, cell-specific anti-cancer mechanism with synergistic activity, and possible pharmacokinetic parameters of Genistein as a potential alternative therapeutic option for the treatment of BC.

Keywords: Genistein; Estrogen receptor; BRCA-1; Cell cycle; Tumor suppressor gene; Molecular pharmacology; Synergistic activity.
1. Introduction:

Breast cancer (BC) is considered a major public health concern among women in health science. BC is responsible for 30% of all cancers in females and is the world's second leading cause of mortality [1]. Although they're diverse risk factors, which can increase the possibility of developing BC including aging, sex, gene mutations, family history, and unhealthy lifestyle, [2], abnormal hormones namely estrogen play an effective role in BC progression [3]. There are currently no established treatments are available, and having some important side effects including-reduction of blood cells, [4], sore throat, hair loss, ulcers, fatigue, nausea, change in taste, appetite loss, constipation, diarrhea, change in skin color, and changes of several hormonal levels [5] and some limitations of existing treatment are high cost, less effective, allergic reaction [6]. Multidrug-resistant (MDR) tumors are the major limitation of conventional treatment, leading to increased cancer-related deaths. Numerous drug molecules likely- anthracyclines (daunorubicin, doxorubicin, mitoxantrone, and epirubicin), taxanes (docetaxel, paclitaxel), and capecitabine were previously used successfully, but now these drugs are becoming resistant to cancer patients [7]. So, the modern medical emphasis is on preventing and treating breast cancer with natural nutritional components [8] [9] [10]. Under the pharmaceutical compounds-phytoestrogens are a natural dietary component with having potent anti-cancer activity against multiple cancer; most importantly, ovarian, prostate, as well as breast [11][12].

One of the major soy phytoestrogens is Genistein (Gen), which has been described as a breast cancer preventive factor [13]. Soy-based foods, for example-soy cheese, and soy drinks i.e., soy milk with soy-based beverages, are the plentiful sources of Gen; of late, the amount of Gen content within mature soybeans has shown from 5.6 to 276 mg/100 g, but 81 mg/100 g is an average content [14], where regular daily intake of this isoflavones among adults in China, and Japan is about 25–50 mg which is several-folds higher than the consumption of these compounds by women in the western countries which accounted for less than 3 mg [15]. Gen has a structural similarity to 17β-estradiol and it binds to estrogen receptor ER-β compare to ER-α [16][17]. Diverse studies suggested the Gen can directly inhibit the cell cycle [18], induce cellular apoptosis process [19][20], suppressed metastasis [21] angiogenesis [22], inhibit inflammation [23], reduce oxidative stress, inhibit tumor growth, and mammosphere formation [24] for both estrogen-responsive, as well as non-responsive BC cells in vitro and several animal models.
Furthermore, this phytoestrogen has several synergistic effects, where Gen can enhance the efficacy of breast cancer therapy along with reducing chemotherapy drug resistance [25]. Major Synergistic effect of Gen with Doxorubicin, Tamoxifen, Trastuzumab, and so on; of later, their activity rely on dose concentration [26][27][28]. Consequently, Gen effects on markers of cancer risk, which were observed from the numerous clinical studies in humans both in vitro and in vivo clinical investigation respectively [29][30][31][32][33]. Genistein safety measurement and future predictions were discovered by using in silico assessment. It possesses a better bioavailability score of 0.55. Similarly, Gen is highly hydrophobic and absorbed by human intestinal, with permeable to Caco-2 cell. In addition, its capacity to be well distributed in the various tissues, also excrete little amount after the metabolism of Gen. Finally, Gen has no severe toxic effect on the human body which was analyzed by the online server, and therefore, it's maintained the drug-like characteristics without any violating of Lipinski, Ghose Veber, Muegge, and Egan rules.

Therefore, in this current investigation, molecular anti-cancer mechanisms of Genistein in multiple BC cells were analyzed, mechanism of resistance drug recovery with synergistic effects, clinical trials, and pharmacokinetics was predicted using Schrodinger’s QuickPro modules and online accessible server admetSAR, SwissADME, and pKCSM for the better understanding of Genistein and breast cancer treatment.

2. Molecular pharmacology of Genistein in breast tissue

Genistein is a natural phytochemical that belong to phytoestrogen and possesses a similar structure to estrogen and most interestingly has both mimic and antagonize estrogen effects, at the same time it inhibits the BC cell proliferation at high concentrations [34]. Estrogen receptor-mediated growth of BC cells by Gen is concentration-dependent. T. T. Y. Wang et al. summarized that Genistein stimulated growth at lower concentrations (10^{-8}~10^{-6} M), but Gen inhibited cancer cell growth at higher concentrations (>10^{-5} M) [35]. There are two types of estrogen receptors [36]. Genistein has a similar structure both of these types of receptors but binds to estrogen receptor-β (ER-β) with higher affinity compared to estrogen receptor-α (ER-α)[16][17].
In the case of estrogen receptor-α, Genistein acts as an antagonist. So, Genistein-mediated anti-cancer activity is involved in suppressing the expression and activity of estrogen receptor-α. E. J. Choi summarised that Genistein regulates cell proliferation with apoptosis via ER-α dependent pathway in MCF-7 breast cancer cells through the underline mechanism of downregulating Cyclin D1 and up-regulating Bcl-2/Bax (full elaboration add here) ratio at the dose of 50 μM [37]. But in ER-β, Gen plays as an agonist activity and increases the receptor activity. Therefore, ER-β dependent anti-cancer activity of Genistein mediated by activating the receptor and potentiate chemotherapy efficacy to treat cancer treatment [38]. H. Jiang state that ER-β1 increased the anti-cancer efficacy of Gen in MDA-MB-231, MCF-7, cells, and in BALB/c mice by inhibiting cell proliferation through arresting cells in G2/M and G0/G1 phase, which led to cell cycle blockade at the dose of 10^{-6}-10^{-4} mol/l [39]. It has also been found to bind with the estrogen-responsive G protein-coupled receptor-30 (GPR-30) or G protein-coupled estrogen receptor-1 (GPER-1) [40], and inhibit cell proliferation [41]. HER-2 is an essential biomarker in breast cancer overexpressed in around 20–30% of breast cancer tumors [42]. So, regulating the HER-2 is a significant factor in BC treatment. M. S. Sakla et al. summarised that Genistein at > or =1 microM inhibited proto-oncogene of HER-2, and subsequently followed the HER-2 protein expression, phosphorylation, and promoter activity through an ER-independent mechanism in BC cells as delaying tumors onset in transgenic mice [43]. Therefore, GPR-30 is a potent alternate to ER that causes BC and can be a target in breast cancer cell treatment [44]. Kim GY et al. summarized that Genistein suppresses the GPR-30 activation in BRCA-1 mutated BC cells, resulting in G2/M phase arrest mediated by suppressing Akt phosphorylation [45]. Furthermore, Gen is a naturally occurring protein tyrosine kinase (PTK) inhibitor that is hypothesized to be responsible for the lower rate of BC observed in Asian women consuming soy. T. Akiyama et al. reported that Gen scarcely inhibited the enzyme activities of threonine- and serine-specific protein kinases like cAMP-dependent protein kinase, Ca2+/phospholipid-dependent enzyme protein kinase C, and phosphorylase kinase, and this mechanism are mediated through phosphorylation of the EGF receptor [46].
3. Cell-specific molecular mechanism of Genistein mediated anti-breast cancer activity *in vitro*

Cancerous cell lines derived from humans are critical models for cancer biology research in laboratories and determine the therapeutics advantage of anti-cancer agents [47]. Anti-cancer activity of phytochemicals is cell-specific, where one phytochemical is effective in only one or more than one cell line. This may be the difference in the cell components system. In this section, cell line-specific anti-cancer activity of Genistein is summarized in Fig 01.

**Figure 01. Graphical overview of the anti-cancer mechanisms of Genistein:** Genistein induces apoptosis through mitochondrial mediated classical caspase-dependent pathway with modulating Bcl-2 family proteins. It induces cell cycle arrest by modulating the cycle regulatory proteins. It inactivates signaling pathways, namely PI3K/AKT pathway, as well as MAPK (ERK1/2) pathways. Formononetin also modulates several miRNA expressions as well as suppresses cell migration, invasion, and angiogenesis and regulate epigenetics control.
3.1. Genistein effect on MCF-7 BC cell

According to R. F. Prietsch et al., Gen (0.01-100µm) promoted apoptosis via mediating the autophagy-dependent mechanism along with increasing the ratio of Bax/Bcl-2 and inhibit the oxidative stress of cancer progression through changing the expression of antioxidant enzymes. For example, Gen down-regulates the thioredoxin reductase enzyme (TrxR) and up-regulates the expression of Glutathione peroxidase (GPx) [48]. X. Liu et al. summarized that Genistein (5-20µm) induces apoptosis through the mitochondrial-dependent pathway by decreasing the Bcl-2/Bax ratio, increasing tumor suppressor gene P73 expression and ATM phosphorylation with G2/M phase arrest permanently [49]. Similarly, Genistein (50-200µm) hinders the cellular growth and induces the apoptosis mediated pathway by following the downregulation of Bcl-2 protein, as well as upregulation of the Bax, together with decreasing the cyclin D1 expression in MCF-7 breast cancer cell [37]. At low concentration Gen (1µM) stimulate cell proliferation but at higher concentration (25µM) induces apoptosis mechanism by up-regulating of the CDKN1A and p53 responsive gene and down-regulating the CCNG1 GADD45A, B NF-kappa B, BCL-2, and TNFR, ESR1, NCOA2, and NCOA3 [50]. Another research investigated that Gen (50µM) highlights apoptosis by up-regulating Poly-(ADP-ribose)-polymerase,p53, and down-regulating Bcl-2/Bax protein [26]. In vitro study by M. L. De Lemos investigated that Gen (10µM) induces apoptosis by breaking plasma membrane breakdown, nuclear membrane, and up-regulating pS2 expression [27]. A late study reported that Gen (100 µM) induces programmed cell death and suppresses cell growth by up-regulating Caspase 7, apoptosis signalling kinase-1, ADP ribose, and p38 dependent mitogen protein kinase [51].

Inhibition of metastasis is common in breast cancer treatment. In vitro study demonstrated that Gen (3.125-12.5µM) decrease metastasis with increasing GSTP1 and RARβ2 gene expression and activity [52]. Shon et al. concluded that Gen (50µM) suppressed angiogenesis by downward COX, Topoisomerase II enzyme and TPA, EROD protein [53], while Gen (1-10 µg/ml) inhibit angiogenesis and cell mutation by decreasing Tyrosine kinase, Ribosomal S6 kinases, and DNA topoisomerases I, II [54]. In vitro study, Genistein (10nm-10µm) lowered cell proliferation via mitochondrial-dependent pathway by reducing Fis1 (mitochondrial fission), and Opal (mitochondrial fusion) mRNA expression [55], while (4-10mol/l) of Gen inhibits cell proliferation by downregulating the cyclin D1 and arresting the cell cycle G0/G1 phase, resulting
in blockage of the cell survival by H. Jiang et al. [39]. J. Chen et al. reported that Gen (5-100µm) inhibited the proliferating cells by inducing apoptosis through IGF-1R-PI3 K/Akt-mediated pathway inactivation and up-regulating Bax/Bcl-2 ratio [56]. Furthermore, it has been shown that Genistein (5-30µm) inhibits BC cell growth, proliferation and promotes apoptosis by following the downregulation of the Hedgehog–Gli1 signaling pathway by decreasing the mRNA level of Smo and Gli1[57]. Marik et al. also find similar result, Gen (0.1 µM) at low concentration stimulate Cancer progression, but Gen (20µM) at high concentration inhibits cell proliferation by down lifting mRNA expression of ER-α protein and arrest the cell cycle at the G2/M phase [34]. Chinni et al. examined that Gen (100µM) inhibits cell proliferation by downregulating Akt mediated signaling pathways, telomere length, and overexpression of cyclin-dependent kinase inhibitor p21WAF1 [58].

An early study demonstrated that Gen (50µM) inhibits tumor growth with apoptosis inductions by increasing Ca²⁺dependent proapoptotic proteases, μ-calpain, and caspase-12 [59]. On the other hand, Liao et al. showed that Gen (100µM) inhibits cell growth alongside decreasing Paclitaxel-induced tubulin Polymerization, Bcl-2, Cyclin B1, and CDK2 kinase, leading to cell cycle arrest at G2/M phase [60]. Chen et al. showed that Gen (50-100µM) inhibits cell division through uplifting HSP protein activity and down lifting SRF mRNA, RAG-1, DOC 2 expression [61]. In breast cancer stem cells, Genistein (40nm -2µm) inhibits mammosphere formation by suppressing PI3K/Akt signaling with up-regulating the PTEN expression[24]. Similar result found by Y. Liu et al. confirmed that Genistein (40nm-2µm) inhibits mammosphere formation and induces differentiation by activating PI3K/Akt and MEK/ERK signaling in a paracrine manner increases E-cadherin mRNA expression by reducing the ratio of CD44+/CD24-/ESA in MCF-7 breast cancer cell [62]. Genistein (1µm) induces an anti-cancer effect through up-regulating pro-inflammatory genes, i.e pS2 and COX2, and downregulating anti-inflammatory gene expression i.e., TFGβ and PPARγ in MCF-7 breast cancer cell [63]. Furthermore, Kazi et al. appeared that Gen (50-200µM) hold back cancer progression by up-regulating IκB-α, p27Kip1 level, and downregulating proteasomal chymotrypsin-like activity, CDKs [64]. Epigenetics regulation by Gen (60-100µm) is mediated by diminishing DNA methylation levels, DNMT1 expression, DNA methyltransferase enzyme activity. However, this reduction of DNA methylation occurs in the promoter region of multiple Tumor suppressor genes (TSGs) such as
Adenomatous polyposis coil (APC), Ataxia telangiectasia mutated (ATM), phosphatase- and tensin homolog (PTEN), mammary serpin peptidase inhibitor (SERPINB5) [65].

3.2. Genistein effect on MDA-MB-231 BC cell

Recently an experimental conducted by X. Liu et al. Gen (5-20µm) induces apoptosis through the mitochondrial-dependent pathway by reducing the Bcl-2/Bax ratio and growth inhibition with decreasing expression of mutant p53 and increasing the expression of p73, leading to the activation of G2/M phase arrest and ATM/Cdc25C/Chk2/Cdc2 checkpoint pathway [49]. Genistein induces the apoptosis process and directly inhibits the growth of cells through the prevention of NF-κB activity by Notch-1 pathway and with downregulating cyclin B1, Bcl-xL, and Bcl-2 expression, resulting in the arrest of the cell cycle at G2/M phase at 5-20µm [66], while at 5-50µm, this phytochemicals induces apoptosis by targeting the endogenous copper ion, reducing Cu(II) to Cu(I) through the production of reactive oxygen species (ROS) [67]. Before that, in vitro study by K. Dampier et reported that Gen (10µM) induces apoptosis and inhibits the rapidly proliferating cells, cell cycle arrest at G2 phase with degrading proto-oncogene c-fos, prohibited protein-1 (AP-1), and also ERK activity [68]. Another research by X. Yang et al. demonstrated that Gen (50µM) highlights apoptosis by up-regulating Poly-(ADP-ribose)-polymerase, activating p53, and downregulating Bcl-2/ Bax protein [26].

In the case of angiogenesis, Mukund et al. explained that Genistein (100µm) reduced angiogenesis by blocking the transactivation of downstream HIF-1α effectors, e.g., VEGF, leading to the reduction of hypoxia-inducible factor-1α expression in MDA-MB-231 BC cell [22]. Furthermore, 1-10 µg/ml of Gen suppressed angiogenesis and cell mutation by decreasing tyrosine kinase, Ribosomal S6 kinases, and DNA topoisomerases I, II [54], while at 50µM concentration decreased angiogenesis with inhibiting cell division through the underline mechanism of downregulating COX, Topoisomerase II enzyme, and TPA, EROD protein activity [53]. Followed by angiogenesis, Gen (15-30 µM) [21], (5-20 µM) [20] obstructs cancer cell migration and invasion respectively through down lifting levels of CDKs, Tyrosine kinase, DNA topoisomerase II, and Paracrine stimulation and decreasing MEK5, ERK5, Phospho-ERK5, NF-κB/p65, and Bcl-2/ Bax. Another study done by O. C. Kousidou et al. reported that Gen (35-100µM) progresses slowdown invasiveness by decreasing MMP gene expression, PTKs activity, and glucose uptake rate leading to phagocytosis cancer cell [69]. Apart from this,
inhibition of cell viability through decreasing the DNA methyltransferase activity, DNMT1 expression and affecting the expression of TSGs likely- APC, ATM, PTEN, SERPINB5 by at 60-100µm of Genistein [65]. Another recent study by D. G. Pons et al. summarized that Genistein (1µm) causes a considerable decrease in cell viability through the mitogen-dependent protein kinase pathway that can promote apoptosis [63].

In MDA-MB-231, BC cell growth controlling is a significant target for Genistein. Gong et al. state that Gen (5-50 µM) inhibits cell growth by partly inducing apoptosis via downregulation of the Akt, NF-κB cascade pathways in vitro study [70]. Another in vitro analysis displayed the cell growth inhibitory activity was evident by Genistein (2.5-400µm) through the up-regulation of two crucial TSGs e.g. p21WAF1 (p21), p16INK4a (p16), and downregulation of two tumor-promoting genes like- c-MYC, and, BMI1, ultimately inhibit cancer progression [71]. Y. Fang et al. concluded that Genistein (40µm) inhibits cellular growth via following the activation of DNA-dependent damage response and ATR signaling pathway with activating the BRCA-1 complex, inhibiting cohesion complex and increasing phosphatide, which is distributed among CDK1, CDK2, and CDK3[72]. Recently it was established that Genistein (1000ppm) suppressed the tumor growth by cell cycle regulating via maintaining the expression level of the cyclin D1 protein, leading to G0/G1 phase arrest, which causes cell cycle blockage[39]. Subsequently, T. Rajah et al. summarised that Gen (10-100 µM) inhibits tumor growth by down-regulating MEK5, pERK5, and NF-κB proteins [73].

In the case of cell proliferation, low dose Genistein (10µm) slightly inhibited cell proliferation by reducing the level of P-STAT3/STAT-5 ratio[55]. In comparison, at a higher dose of 20-40µm, it significantly prevents cell proliferation by inducing apoptosis by suppressing Skp2 expression by up-regulating the tumor suppressor genes, i.e. p21, p27, resulting in G2/M phase arrest [74]. Li et al. investigated that Gen (5-20 µM) inhibits differentiation with the arresting at G2/M phase by decreasing Cdk1, CyclinB1, Cdc25C, c-Jun, and c-Fos level [18]. Gen can also play a role in MDA-MB-231 specific by inhibiting mammosphere formation. A lower dose of Genistein (2µm) prevents the mammosphere formation through PI3K/Akt signaling by increasing the PTEN expression[24], while at a higher dose Genistein (40nm-2µm) prevents the formation of mammosphere cells and promotes differentiation through the PI3K/Akt and MEK/ERK signaling pathway by reducing the CD44+/CD24-/ESA ratio and increasing E-cadherin mRNA
expression [62]. Finally, Gen (50 µM) impeded primary tumor formation by down-regulate the chelator neocuproine, Bcl-2/ Bax, at the same time up-regulate the caspase-3 pathway [67].

3.3 Effect of Genistein on T-47D breast cancer cell:

V. Mukund et al. summarize that Genistein (50µm) lowers angiogenesis by preventing the transactivation of downstream HIF-1α effectors such as VEGF, resulting in the lessening the expression of hypoxia-inducible factor-1α in T-47D type of breast cancer cell lines [22]. Cell proliferation efficacy was evident by Genistein (10nm) with apoptosis induction through the mitochondrial-dependent pathway via up-regulating the cytochrome c oxidase activity and downregulating the ATP synthase/cytochrome c oxidase ratio [55]. Genistein at the 1nm-100µm inhibits cell proliferation through ERK1/2 mediated signaling by the downregulation of phosphorylated p90RSK [75], while 10µM of Gen induces apoptosis and inhibits cell proliferation through degrading proto-oncogene c-fos levels and prohibited Protein 1 (AP-1) and ERK expression [68]. Another in vitro study by T. T. Rajah revealed that Gen (10-100 µM) inhibits Cell proliferation and Tumor growth by downregulating MEK5, pERK5, and NF-κB proteins [73]. Additionally, Marik et al. demonstrated that while a low concentration of Gen (0.1 M) promotes cancer progression, a high concentration of Gen (20 M) inhibits cell proliferation by reducing ER-messenger RNA transcription and arresting the cell cycle at the G2/M phase [34]. According to A. M. Sotoca et al. summarized that Gene (500nm) inhibits cell growth and induces apoptosis by activating cytoskeleton restructuring that results in interaction among integrins, focalized adhesion kinase and CDC42 that leads to cell cycles arrest in T-47D breast cancer cell line [76]. while according to D. G. Pons et al., Gen (1µm) causes a significant decrease in cell viability by increasing Sirt1, TGFβ, PARRγ and decreasing IL-1β expression in T-47D breast cancer cell [63].

3.4 The Actions of Genistein on HCC1395 breast cancer cell:

W. Y. Lee et al. demonstrated that Genistein (1-200µm) inhibits invasion and metastasis through the upregulation of TFPI-2, ATF3, DNMT1, MTCBP-1 genes expression and downregulation of MMP-2, MMP-7, CXCL12 genes, leading to arrest G2/M phase and therefore, reduces cell viability [77].
3.5 The Actions of Genistein on HCC38 breast cancer cell:

M. G. Donovan stated that Genistein (4-10ppm) inhibits cell growth by increasing the BRCA1 protein level and reducing the CpG methylation, consequently decreases the AHR binding at BRCA1 [78].

3.6 The Actions of Genistein on Hs578t breast cancer cell:

According to C. De La Parra et al., Genistein (1-50µm) inhibits cell viability and induces apoptosis through the downregulation of mir-155, resulting in upregulation of casein kinase, FOXO3a, p27, PTEN expression and reducing the level of β-catenin [79].

3.7 The Actions of Genistein on DD-762 Cell and Sm-MT C breast cancer cell:

Nakagawa et al. appraised that Gen (7-274.2µM) inhibits cell proliferation by up-regulating caspase-3 protein activity [80].

3.8 The Actions of Genistein on BT-474 breast cancer cell

Genistein low concentration (1 µM) can promote cancer but at high concentration (50 µM) inhibits the cell division with downregulating Tyrosine kinase, HER2 activation, and MAPK pathway [43]. A similar study approached the same result on the BT-474 cell line. Gen (3.125-25 M) inhibits cell replication and arrests the cell cycle in the G2/M phase, as well as inhibiting the expression of EGFR, HER2, and ER-alpha [81].

3.9 The Actions of Genistein on BT20 breast cancer cell

Cappelletti et al. revealed that Gen (15-30 µM) inhibits metastasis through down lifting levels of CDKs, Tyrosine kinase, DNA topoisomerase II, and Paracrine stimulation BT20 Cell Line [21].

3.10 The Actions of Genistein on 21PT breast cancer cell

Marik et al. demonstrated that while Gen (0.1 M) stimulates cancer progression, Gen (20 M) inhibits cell proliferation by decreasing ER-messenger RNA expression and arresting the cell cycle at the G2/M phase in the 21PT Cell Line [34].
3.11 The Actions of Genistein on 184-B5/HER breast cancer cell

Katdare et al. showed that Gen (2.5-10 µM) impedes the cell cycle by inducing apoptosis by increasing the P16INK4a gene and decreasing HER-2/neu, Tyrosine kinase [82].

3.12 The Actions of Genistein on MCF-10A, MCF-ANeoT, MCF-T63B breast cancer cell

An early study showed that Gen (1-10 µg/ml) obstructs angiogenesis and cell mutation by decreasing the expression of DNA topoisomerases I and II, Ribosomal S6 kinases and Tyrosine kinase [54]. Overview of anti-cancer activities were tabulated in Table 01.

Table 01. tabular representation of in vitro anti-breast cancer activity Genistein

| Target | Pharmacological interaction | Type of study (in vitro and in vivo) | Dose | Molecular mechanism | Molecular target | Ref |
|--------|-----------------------------|-------------------------------------|------|---------------------|------------------|-----|
|        | In vitro (MCF-7)            | 10-100µm                           | ↑Apoptosis ↓Cell proliferation | ↓IGF-1R-PI3 K/Akt pathway ↓Bcl-2/Bax mRNA | [56] |
| ER-α   | Antagonist                  | In vitro (MDA-MB-231 and T-47D)    | 50-100µm | ↓Angiogenesis | ↓VEGF ↓HIF-1α expression | [22] |
| ER-α   | Agonist                     | In vitro (MCF-7, T47D, and MDA-MB-231) | 10-10µm | ↓Cell proliferation ↓Mitochondrial activity | ↓Opa1, Fis1 ↓cytochrome c oxidase ↓ATP synthase/cytochrome c ratio ↓P-STAT3/STAT-3 ratio | [55] |
|        | In vitro (MCF-7)            | 5-30µm /20-50 mg/kg                | ↑Apoptosis ↓Cell growth and proliferation | ↓Endogenous copper ion ↑generation of reactive oxygen species (ROS) | [67] |
|        | In vitro (MDA-MB-231, and MDA-MB-468) | 5-50µm | ↑Apoptosis | ↓mRNA expression of DNMT1 ↓DNA methylation in tumor suppressor Genes | [65] |
|        | In vitro (MCF-7 and MDA-MB-231) | 60-100µm | ↓Cell viability | ↑BAX/Bcl-2 ratio ↑Thioredoxin reductase (TrxR), ↑Glutathione peroxidase (GPx) | [48] |
|        | In vitro (MCF-7)            | .01-100µm                          | ↑Apoptosis ↑Cell cycle arrest | ↑Arrest G2/M phase ↓NF-kB, Notch-1 pathway ↓cyclin B1, ↓Bcl-2 and Bcl-xL | [66] |
|        | In vitro (MDA-MB-231)       | 5-20µm                             | ↑Apoptosis ↑Cell cycle arrest | ↓Skp2 expression ↑Arrest G2/M phase ↑p21, p27 | [74] |
|        | In vitro (MDA-MB-231 and SKBR3) | 20-40µm | ↓Cell proliferation ↑Apoptosis ↓Metastasis | | |
|        | In vitro (MCF-7)            | 75-200µm                           | ↓Cell growth | ↑miR-23b ↑Target PAK2 gene | | [83] |
| In vitro | (MDA-MB-231) | 2.5-400µm/250mg/kg | ↓Cell growth | ↑p21WAF1 (p21), ↑p16INK4a (p16) ↓BMI1, c-MYC | [71] |
|---|---|---|---|---|---|
| ER-α Agonist | ER-β Antagonist | In vitro | (MCF-7 and MDA-MB-231) | 5-20µm | ↑Apoptosis ↑DNA damage ↑ Arrest G2/M ↑ATM, Bax, p73 ↓Bcl-2, ↓Bcl-2/Bax rate, mutant P53 | [49] |
| ER-α Agonist | ER-β Antagonist | In vitro | (MCF-7, MDA-MB-435, and Hs578t) | 1-50µm | ↓Cell viability ↑Apoptosis ↓miR-155 ↓β-catenin ↑Casein kinase, ↑FOXO3a, p27, PTEN, CK1α | [79] |
| In vitro | (MDA-MB-231) | 40µm | ↓Cell growth | ↑ phosphopeptide ↓ Cohesin complex ↑DNA damage response pathway ↑ BRCA1 ↑ ATC signaling pathway | [72] |
| ER-α Agonist | ER-β Antagonist | In vitro | (MCF-7 and MDA-MB-231) | 40 - 2µm | ↓ Mammosphere formation ↑PTEN expression ↓PI3K/Akt signaling | [24] |
| ER-α Agonist | ER-β Antagonist | In vitro | (MCF-7, T47D and MDA-MB-231) | 1µm | ↓Cell viability ↓Cell proliferation ↑ROS, pS2, Sirt1, COX2 ↓IL-1β, TFGβ, PPARγ | [63] |
| ER-α Agonist | ER-β Antagonist | In vitro | (T47D) | 500nm | ↓Cell growth ↑Apoptosis ↑Cytoskeleton remodeling ↑integrins, focal adhesion kinase, CDC42 ↑Arrest cell cycle | [76] |
| ER-α Antagonist | In vitro | (T47D) | 1-100µm | ↓Cell proliferation ↓ERK1/2, p90RSK | [75] |
| ER-α Antagonist | In vitro | (MCF7, UACC3199, and HCC38) | 4-10ppm | ↓Cell growth ↑ Activate BARCA-1 ↓ CpG methylation ↓AHR activity | [78] |
| ER-β Agonist | In vitro | (MCF-7 and MDA-MB-231) | 10⁻⁶ mol/l- 10⁻⁴ mol/l /100-1000ppm | ↓Cell proliferation ↑Cyclin D1 ↑Arrest G0/G1 phase | [39] |
| ER-α Agonist | ER-β Antagonist | In vitro | (MCF-7 and MDA-MB-231) | 40nm-2µm | ↓Mammosphere formation ↑Differentiation ↑CD44+/CD24-/ESA ratio ↑PI3K/Akt, MEK/ERK signaling ↑E-cadherin expression | [62] |
| ER-α Antagonist | In vitro | (MCF-7 and UACC-3199) | 0.5-20µm | ↓Cell growth ↑BRCA-1 ↓CpG methylation, ↓Cyclin D1, ↓DNMT-1 ↑AhR ↑CYP1A1 | [84] |
| ER-α | Antagonist | 
|------|----------|----------|----------|----------|----------|----------|----------|
| ER-β | Agonist | In vitro (HCC1395) | 1-200µm | ↓Cell viability | ↓Invasion | ↓Metastasis | ↑TFPI-2, ATF3, DNMT1, MTCBP-1 | ↓MMP-2, ↓MMP-7, CXCL12 | ↑Arrest G2/M phase | [77] |
| ER-α | Antagonist | In vitro (SUM1315MO2) | 1-100µm | ↓Cell proliferation | ↑ER-β expression | ↑Bcl-2, ↑Bax | ↓Cyclin D1 | ↓Bcl-2/Bax ratio | [85] |
| ER-α | Antagonist | In vitro (MCF-7) | 50-200µm | ↓Cell growth | ↑Apoptosis | ↓Bcl-2, ↑Bax | ↓Cyclin D1 | ↓Bcl-2/Bax ratio | [37] |
| ER-α | Agonist & Antagonist | In vitro MCF-7 | 1-25 µm | ↑Apoptosis | ↓Cell proliferation | ↑CDKN1A, TNF-α p53 responsive gene | ↓CCNG1 and GADD45A | ↓BCL-2, Bcl-3 and NF-kappa B | [50] |
| ER-α | Antagonist | In vitro MDA-MB-435 | 750 µg/g | ↑Apoptosis | ↓Metastasis | ↓c-fos levels, Protein-1 (AP-1) activity, (ERK) activity | ↑Poly-(ADP-ribose)-polymerase, p53 | ↓Anti-apoptotic protein | [86] |
| ER-α | Antagonist | In vitro MCF-7, ZR-75.1, T47-D, MDA-MB 468, MDA-MB 231 and HBL | 1-10 µm | ↑Apoptosis | ↓Cell proliferation | ↓c-fos levels, Protein-1 (AP-1) activity, (ERK) activity | ↑Arrest at G2 phase | [68] |
| ER-α | Antagonist | In vitro MCF-7 and MDA-MB-231 | 50 µm | ↑Apoptosis | ↓Cell cycle kinetics | Poly-(ADP-ribose)-polymerase, p53 | ↓Anti-apoptotic protein | [26] |
| ER-α | Antagonist | In vitro MCF-7 and MDA-MB-231 | 50 µm | ↑Apoptosis | ↓Cell division | ↓EROD, TPA | ↓Cyclooxygenase, Topoisomerase II, Tyrosine Kinase. | [53] |
| ER-α | Antagonist | In vitro MDA-MB-468 | 25-100 µm | ↓Cell cycle kinetics | ↑Apoptosis | ↓Cell proliferation | ↑Arrest at G2/M phase | ↑Nuclear membrane breakdown during G2/M transition | ↓DNA synthesis | [87] |
| ER-β | Antagonist | In vitro MCF-7 | 0.001-10 µm | ↑Cells underwent apoptosis | ↑Plasma membrane breakdown | ↑Nuclear membrane breakdown | ↓pS2 expression | [27] |
| ER-β | Antagonist | In vitro MCF-7 and MDA-MB-231 | 10-100 µm | ↑Apoptosis | ↓Cell division | ↓PTK, Akt, FAK, ErbB-2, and Bcl-2 | ↓Topoisomerase II, Tyrosine kinase | ↓Osteoclast activity | [46], [69], [88] – [95] |
| ER-α | Agonist Antagonist | In vitro BT-474 | 1-50 µm | ↑Apoptosis | ↓Cell division | ↓Tyrosine kinase, HER2 activation, MAPK pathway | [43] |
| ER-α | Antagonist | In vitro MDA-MB-231 | 0.5-15 µm | ↓Cell cycle kinetics | ↑Apoptosis | ↓Anti-growth signals protein | ↓Connexin phosphorylation blocks the homeostatic regulators | [96] |
| ER-α | Antagonist | **In vitro** MDA-MB-231, BT20, T47D, and ZR75.1 | 15-30 µm | ↑ Cell underwent apoptosis ↓ Migration | ↑ Arrest at G2/M phase ↓ Tyrosine kinase ↓ DNA topoisomerase II ↓ Paracrine stimulation | [21] |
| ER-α Agonist & Antagonist | **In vitro** MDA-MB-231 & T47D | 10-100 µm | ↓ Decrease cell proliferation ↓ Tumor growth | ↓ MEK5, pERK5, NF-κB proteins | [73] |
| ER-α | Antagonist | **In vitro** AS-4, NEO & BG-1 | 25-150 µm | ↑ Inhibit cell proliferation ↑ Induce apoptosis | ↓ Cytotoxic effect in AS4, Tyrosine kinase, Topoisomerase II ↑ Ubiquitin E3 ligase | [97] |
| ER-α | Antagonist | **In vitro** MDA-MB-231 | 5-20 µm | ↑ Trigger apoptosis ↓ Invasion | ↑ Trigger G2/M cell cycle arrest ↓ Cdk1, CyclinB1 and Cdc25C ↓ c-Jun and c-Fos | [18] |
| ER-α | Agonist & Antagonist | **In vitro** MCF-7 | 50 µm | ↓ Tumor growth ↑ Induces apoptosis | ↑ Ca²⁺ dependent proapoptotic proteases ↑ µ-calpain, and caspase-12 | [59] |
| ER-β Agonist | **In vitro** MDA-MB-231 | 20-80 µm & 750 µg/g | ↓ Cell growth ↑ Tumor formation in vivo | ↑ Cell cycle blocked at G2/M | [98] |
| ER-α | Antagonist | **In vitro** MCF-7 and MDA-MB-231 | 100 µm | ↑ Induced apoptosis ↓ Cell proliferation | ↑ Arrest at G2/M phase ↓ Paclitaxel-induce tubulin Polymerization ↓ Bcl-2 phosphorylation ↓ Cyclin B1 and CDC2 kinase | [60] |
| ER-α | Antagonist | **In vitro** BT-474 | 3.125-25 µm | ↑ Induction of apoptosis ↓ Duplication | ↓ HER2 and ER-alpha ↑ Cell cycle arrest at S and G2/M ↑ Expression of surviving, EGFR | [81] |
| ER-α | Antagonist | **In vitro** MDA-MB-231, MCF-7, and MCF-12A | 35-100 µm | ↓ Cell invasiveness ↓ Cell cycle ↑ Phagocytosis | ↓ Protein tyrosine kinase pathway ↑ Arrest at G2/M phase ↓ MMP genes ↓ Glucose uptake | [69] |
| ER-α | Antagonist | **In vitro** MCF-7 | 100 µm | ↑ Induce Apoptosis ↓ Cell Growth | ↑ Caspase 7 and poly (ADP ribose) polymerase ↑ Apoptosis signalling kinase 1 ↑ p38 MPK | [51] |
| ER-α | Antagonist | **In vitro** MDA-MB-231 | 5-50 µm | ↑ Induce Apoptosis ↓ Cell cycle | ↑ Apoptosis related genes ↓ Akt and ↓ NF-κB ↓ EGF | [70] |
| ER-α | Antagonist | **In vitro** MCF-7 | 50-200 µm | ↓ Cell invasion ↑ Apoptosis ↓ Cancer progression | ↓ Proteasomal chymotrypsin-like activity ↓ CDKs inhibit by p27Kip1 ↑ IκB-α level | [64] |
| ER-α | Antagonist | **In vitro** MCF-7 | 50-100 µm | ↓ Cell division ↓ Cancer progression | ↓ SRF, RAG-1, DOC 2 ↑ Arrest at G2/M phase ↑ Heat shock protein 105 (HSP) mRNA | [61] |
### Table 1: Effects of Genistein on Breast Cancer Cells

| **ER-α** | **Agonist/ Antagonist** | **In vitro** | **Concentration** | **Effects** | **References** |
|----------|-------------------------|--------------|-------------------|-------------|---------------|
| ER-α     | Antagonist              | MCF-7 and MDA-MB-468 | 3.125-12.5 µm | ↓ primary tumor, ↓ Metastasis, ↑ GSTP1 gene, ↑ RARβ2 gene | [52] |
| ER-α     | Antagonist              | MCF-7         | 1-100 µm          | ↑ Apoptosis, Cell cycle arrest, ↓ CDKs, Akt activity, ↓ Telomere length | [58] |
| ER-α     | Antagonist              | MCF-7, 21PT, and T47D | 0.1-20 µm | ↓ Cell proliferation, ↓ Cancer progression, ↓ ER-α messenger RNA expression, ↑ Er-β, ↑ Cell cycle arrest at the G2-M phase | [34] |
| ER-α     | Antagonist              | MDA-MB-231, and MDAMB-468 | 50 µm | ↓ Primary tumor, ↑ Apoptosis, ↓ Chelator neocuproine, ↓ Bcl-2, ↑ Bax and caspase-3 | [67] |
| ER-α     | Antagonist              | 184-B5/HER    | 2.5-10 µm         | ↑ Cellular apoptosis, ↓ Cell cycle regulators, ↑ P16INK4a gene, ↓ HER-2/neu, ↓ Tyrosine kinase, ↓ Arrest at S + G2/M phase | [82] |
| ER-α     | Antagonist              | MCF-10A1, MCF-ANeoT, MCF-T63B, MCF-7, MDA-231, MDA-435 | 1-10 µg/ml | ↓ Angiogenesis, ↓ Cell proliferation, ↓ Cell mutation, ↓ Dysplastic and malignant, ↓ Tyrosine kinase, ↓ DNA topoisomerases I and II, ↓ Ribosomal S6 kinases | [54] |

### 4. Mechanism of synergistic activities of Genistein with Chemotherapeutics Resistance Breast Cancer Treatment breast cancer drugs

Except for the strong anti-cancer activity alone, Genistein possesses the synergistic property with many other anti-cancer drugs, which assist it in overcoming the chemopreventive resistance mechanism in breast cancer treatment. The synergistic activity of Genistein can be carried by many anti-cancer drugs such as Doxorubicin, Trastuzumab, Tamoxifen, Trichostatin A, Cisplatin, Capsaicin, Paclitaxel, and vincristine. Tumor cells frequently appear to have a decreased capacity for cytoprotective drug accumulation as a result of the drugs efflux protein (MDR) serving as the pharmacological background for multidrug resistance, these efflux proteins, namely as the BCRP, MRP 1, and P-glycoprotein [99] [100] and the changes in beta-tubulin [100]; It is significant to mention that the multidrug resistance protein (MRP) family has nine members (MRP1–9) [101].

Research study conducted by C.H.M. and colleagues has shown that Genistein inhibited drug accumulation in various types of multiresistant tumor cell lines mediated by non-P-glycoprotein (HT1080/DR4, HL60/ADR, MCF7/Mitox, GLC4/ADR, and SW-1583/2R120) [102]. According
to another research study, genistein, in the form of phytoestrogens, can effectively suppress the breast cancer resistance protein, also known as ABCG2, which is responsible for multidrug resistance activity [103]. Moreover, others research studies have analyzed that the Genistein's ability to autophosphorylate protein tyrosine kinases (PTK), particularly EGF-R, by contesting ATP than other the protein substratum. The genistein antiproliferative effect was also attributable to interfering with the mitogen-activated tyrosine kinase cascade [46].

4.1 Synergistic Properties of Genistein in the treatment of breast cancer

4.1.1 Synergistic Properties of Genistein with Doxorubicin in MCF-7/Adr cell: Doxorubicin is an antibiotic that exhibits no inhibitory effects on Adriamycin resistance breast cancer cell. But the combination of Genistein at 30µmol/l and increasing doses of Doxorubicin possess synergistic effects on MCF-7/Adr cell. Genistein enhances the cytotoxic effect of Doxorubicin and decreases the MCF-7/Adr breast cancer cell's chemoresistance. In addition, Genistein and Doxorubicin induce apoptosis synergistically by decreased expression of Her2/new mRNA and c-erbB2, resulting in cell cycle arrests in MCF-7/Adr breast cancer cell in G2/M phase [25]. Another study by X. Yang et al. reported that Gen (50 µm) with a combination of Doxorubicin slightly induces apoptosis by destroying the plasma membrane of cells and increase Poly (ADP-ribose) polymerase cleavage in MDA-MB-231 and MCF-7 breast cancer cells [26].

4.1.2 Synergistic effect of Genistein with Trastuzumab: Genistein and Trastuzumab synergistically develop the antitumor activity in BT-474 breast cancer cells. C. Lattrich et al. stated that the combination of Genistein (10µmol/l) and Trastuzumab (1/10 µg/ml) enhances the growth-inhibitory effect and reduces viable cell numbers by increasing the ER-β2 expression, which causes an antiestrogenic effect, leading to reducing cell proliferation in ER-α/β-positive, and HER2-overexpressing BT-474 breast cancer cell. Furthermore, both Genistein and trastuzumab reduce cyclin A2 mRNA expression, c-fos, HER2, and induce cyclin D1 expression which suppresses the cell proliferation of BT-474 breast cancer cell [104].

4.1.3 Synergistic Properties of Genistein with Tamoxifen: Tamoxifen is a well-established medicine for treating breast cancer, although its efficacy has been hampered by the development of gemcitabine resistance. In this regard, Gen can improve the efficacy of Tamoxifen. Y. Li et al. described that Genistein enhanced the anti-cancer capacity of Tamoxifen at the dose of 25µm
through the reactivation of ER-α through epigenetic pathways i.e., histone modification, resulting in the reduction of HDAC1 & DNMT1 expression both \textit{in vitro} (ER-α negative MDA-MB-231 breast cancer cell) & \textit{in vivo} leading to inhibits the cell growth and cell viability [28]. On the other hand, D. G. Pons et al., concluded that 1\(\mu\)M Genistein and 10\(\mu\)M Tamoxifen declines the ROS production in T47D, and MCF-7 breast cancer cell and up-regulate the autophagic vacuole formation & PARP protein level and also reduce cell viability, resulting in autophagic cell death only in T47D breast cancer cell [105]. Another early study reported that Tamoxifen, with Gen (1-10 \(\mu\)g/ml), impede angiogenesis and cell mutation by downregulating DNA topoisomerases I and II, Ribosomal S6 kinases, tyrosine kinase, and Cell cycle regulators [54]. Gen also shows a prohibitory effect with Tamoxifen which induces apoptosis by destroying nuclear membrane [27], arrest cell cycle by decreasing expression of HER2 in a dose-dependent manner [81].

4.1.4 Synergistic effect of Genistein with Trichostatin A: Genistein and Trichostatin A act synergistically to inhibit PGR (progesterone receptors) expression (elaboration required), resulting in a significant change in cell growth in ER-positive and ER-negative MCF-7 cells. Breast cancer cell line MDA-MB-231. According to Y. Li et al., the association of Genistein (25\(\mu\)m) and Trichostatin A (100ng/ml) synergistically inducessplatin synergistically decreases the ROS production and enhances antioxidant enzyme i.e the Mn-SOD (Manganese-Superoxide dismutase) & catalase, in MCF-7 & T47D breast cancer cell. Furthermore, Genestein and Cisplatin also synergistically arrest the cell cycle at S phase and cause a drop in subG0/G1 phase, resulting in MCF-7 cells that are apoptotic at (25-50mol/L) concentrations [105].

4.1.5 Synergistic effect of Genistein with capsaicin: The combination of genistein and capsaicin exerted anti-inflammatory and anticarcinogenic effects in MCF-7 cells as well as in vivo 48 weeks female Sprague-Dawley rats by modulating the mitogen-activated protein kinase (AMPK) and COX-2, as well as possibly other mitogen-activated protein kinases [23].

4.1.6 Synergistic effect of Genistein with Paclitaxel and vincristine: Paclitaxel and vincristine both are chemotherapy drugs, also called plant alkaloids. Together with Gen (100 \(\mu\)m), they can suppress cell growth and viability through inhibiting CDC2 and Cycin B1 kinase, and inhibit microtubule polymerization in human MDA-MB-231 and MCF-7 and breast cancer cell line, ultimately cell death by inducing apoptosis via decreasing Bcl-2 phosphorylation without
changing p21, p53, and Bax protein expression [60]. The Table 02 contains a summary of the effects of phytoestrogens in combination with anti-cancer therapies that have been previously described.

Table 02. Summary of the described effects of phytoestrogens in combination with anti-cancer therapies.

| Treatment option | Study type | Effective mechanism | Ref |
|------------------|------------|---------------------|-----|
| Doxorubicin      | In vitro (MCF-7/Adr) | ↓Chemoresistance of tumor cells ↑Cell cycle Arrest G2/M phase ↑Apoptosis ↓Her2/neu mRNA expression ↓c-erbB2 expression | [25] |
|                  | In vitro MCF-7 and MDA-MB-231 | ↑ Destroyed the plasma membrane of cells ↑ Apoptosis in most of the individual MCF-7 cells ↑ Poly (ADP-ribose) polymerase cleavage | [26] |
| Trastuzumab      | In vitro (SK-BR-3, BT-474, and MDA-MB-231) | ↓ Cell proliferation ↑ Cell cycle arrest ↓c-fos, ↓ HER2 | [104] |
| Tamoxifen (TAM)  | In vitro and vivo (MDA-MB-231 female immunodeficiency nude mice) | ↓ Cell growth and Cell viability ↓ HDAC1, ↓ DNMT1 | [28] |
|                  | In vitro MCF-7 | ↑ Destroyed the plasma membrane of cells ↑ Nuclear membrane breakdown ↑ pS2 expression | [27] |
|                  | In vitro BT-474 | ↓ Expression of HER2 and ER-α ↓ Expression of factor and EGFR | [81] |
|                  | In vitro Dysplastic, malignant cells | ↓ Cell growth, proliferation | [54] |
| Trichostatin A   | In vitro (MCF-7, MDA-MB-231 and MDA-MB-157) | ↑ ER-α reactivation, ↑ Histone remodeling ↓ HDACs, DNMTs, PGR expression | [28] |
| (TSA)            | In vitro (MCF-7 and T47D) | ↓ ROS production ↑ MnSOD, Catalase activity ↑ Arrest S and G0/G1 phase | [105] |
| Cisplatin (CDDP) | In vitro MCF-7 MDA-MB-231 | ↓ ROS, LC3-II/LC3-I ratio ↓ cell viability ↑ PARP | [105] |
| Tamoxifen (TAM)  | In vitro (MCF-7 and T47D) | ↑ Putrescine, Spermidine and Spermine ↓ 12-Otetradecanoylphorbol-13-acetate (TPA) ↓ Cyclooxygenase (COX) | [53] |
| Ornithine        | In vitro MCF-7 MDA-MB-231 | ↓ Bcl-2 phosphorylation ↓ Cyclin B1 and CDC2 kinase ↓ Cell viability ↓ Microtubule polymerization | [60] |
| decarboxylase    | In vitro MCF-7 and MDA-MB-231 | ↓ Serum MDA | [106] |
| Lycopene         | In vivo | |
5. Clinical trials

Human clinical trials have confirmed the in vitro and in vivo research findings; in some cases, when consumed at a consistent dose, pure Genistein had no estrogenic effect on breast tissue [29], [30], although others dietary soy supplementation had proestrogenic properties on breast tissue [31]–[33]. Several secondary endpoints were evaluated in a recently published clinical study to determine whether purified Genistein has an effect on the endometrial thickness, vaginal cytology, and breast density (Table 3) [29][93],[94]. Following the implementation of these safety measures, it was possible to identify the potential estrogentic effects of 54 mg/day of purified Genistein as indicators of breast cancer risk in the research participants.

Indeed, while the placebo group maintained a constant endometrial thickness, the Genistein group demonstrated a time-dependent reduction that reached statistical significance during the 36-month follow-up (approximately 12% reduction, P<0.01). Moreover, levels of gene expression of BRCA-1, and 2, breast tumor suppressor genes [109], [110] have been preserved for the three years in the genistein-administered group, while levels of both BRCA-1, and 2 have decreased in the placebo group (Table 3) [29][30]. Genistein also significantly reduced sister chromatid exchanges, implying that it may prevent genotoxicity and subsequent mutagenesis (Table 03) [30].

In this regard, based on the use of Genistein in breast cancer, 2 clinical trials, a phase II study entitled “Gemcitabine Hydrochloride and Genistein in Treating Women with Stage IV Breast Cancer,” and a phase I study entitled “Genistein in Preventing Breast or Endometrial Cancer in Healthy Postmenopausal Women,” have been completed, but the results are not yet published (NCT00244933, NCT00099008). Effects of Genistein on markers of cancer risk observed from human clinical studies was summarized in

Table 03. Table 04: Effects of Genistein on markers of cancer risk observed from human clinical studies (post-menopausal women)
| Subjects (n) | Number/intervention (mg/day) | Study length (months) | Results | Ref |
|-------------|-----------------------------|----------------------|---------|-----|
| 57          | 54g/day genistein (n = 30) or placebo (n = 27) | 12                   | Protect genomic damage | [30] |
| 90          | HRT (n = 30; 1 mg 17 beta-estradiol combined with 0.5 mg norethisterone acetate), Gen (n = 30; 54 mg), or placebo (n = 30) | 12                   | ↑ BMD in the femoral neck and lumbar spine; ↑ serum B-ALP and BGP; | [108] |
| 389         | Placebo (n = 191; 1000 mg calcium and 800 mg vitamin D) or Gen (n = 198; 54 mg Gen, 1000 mg calcium, and 800 mg vitamin D) | 24                   | ↑ BMD at the lumbar spine and the femoral neck; ↑ B-ALP and IGF-1; | [107] |
| 220         | Placebo (n = 111) or isoflavone (n = 109; ~50 mg isoflavones) | 24                   | ↑ breast area ↓ breast density; neither was significant when compared to placebo | [111] |
| 34          | Placebo (n = 17) or soy (n = 17; 100 mg isoflavone, ~76 mg aglycones) | 12                   | ↓ Breast area no change in dense areas or percent breast density No significant changes in any hormone levels | [112],[113] |
| 84          | Placebo (n = 23) or soy (n = 28; 60 mg, ~45 mg isoflavones) | 14 days              | ↓ Nipple aspirate levels of apolipoprotein D; ↑ pS2 | [32],[31] |
| 389         | 54 mg of genistein aglycone daily (n = 71) or placebo (n = 67) | 36                   | unchanged breast density, BRCA1 and BRCA2; ↓ sister chromatid exchange. ↓ pyridinoline, NF-B receptor activator ↑ alkaline phosphatase, IGF-1, and osteoprotegerin | [29] |

6. Safety measurement of Genistein and future prediction

ADMET (absorption, distribution, metabolism, elimination, and toxicity) testing evaluates prospective pharmacological substances, identifying both those with promise and those with significant limitations. ADME/Tox profile of Genistein was predicted using online accessible SwissADME [114](drug likeness properties), pkCSM [115](absorption, distribution, excretion) [115]and server admetSAR [116] (metabolism, toxicity) in silico tools and tabulated in Table 04.
With a good bioavailability score of 0.55, Genistein maintained drug-like characteristics without any violating of Lipinski, Ghose Veber, Muegge, and Egan rules. In drug development, absorption is an important parameter. Our in silico predicted result showed that Genistein is highly soluble in water (-3.376) and absorbed by human intestine, with permeable to Caco-2 cell. After absorption, consequentially comes distribution, another principal descriptor for drug development, which depends numerous factors i.e., steady-state volume of distribution (VDss), blood-brain barrier (BBB) and central nervous system(CNS) permeability, binding to plasma protein, and many more [117]. Our analysis suggested that Gen has the potentiality to have a poor VDss value, but is able to cross the BBB and central CNS properly. Human cytochrome P450 (CYP) isoforms are involved in drug metabolism in the liver. Its inhibition could lead to drug toxicity, drug-drug interactions, and other adverse effects. The predicted metabolic result reported that Genistein is the inhibitor of CYP1A2, CYP2C19, CYP2C9, and CYP3A4 but a non-inhibitor of CYP2D6. Excretion property based on total renal clearance parameter was predicted, where total clearance (logCLtot) of Genistein was 0.151 ml/min/kg. The toxicity profile of Genistein has been predicted based on eye corrosion, hepatoxicity, Skin sanitation, OATP2B1 inhibitor, OATP1B3 inhibitor, OCT2 inhibitor, carcinogenicity, AMES toxicity, hERG I inhibitors, and BSEP inhibitors. Results outlined that Genistein showed toxicity only in the liver and OATP2B1 inhibitor, whereas positive to eye corrosion, hepatoxicity, AMES toxicity, hERG potassium channel inhibition, and carcinogenicity. Anti-cancer activities were determined based on P-glycoprotein and aromatase enzyme inhibition and estrogen receptors modulating affinity. Our analysis revealed that Genistein has a potent affinity to estrogen receptors, aromatase enzyme, but its non-inhibitor P-glycoprotein.

To achieve more information for better bioavailability and drug-likeness of Genistein, detailed information is summarized in Fig 02. The results of the bioavailability radar have been depicted by the lipophilicity: XLOGP3 between −0.7 and +5.0, size: MW between 150 and 500 g/mol, polarity: TPSA between 20 and 130 Å2, solubility: log S not higher than 6, saturation: fraction of carbons in the sp3 hybridization not less than 0.25 and flexibility: no more than 9 rotatable bonds with the colored zone defined the desired physicochemical space for good oral bioavailability indicating that they possess good drug-likeness properties. After the validation of bioavailability, potent drug likeness properties in cancer research models, it can be estimated that Genistein can
be used as an effective anti-cancer natural agent with its synergistic use with other chemotherapeutics for the treatment of breast cancer.

**Table 04. In silico pharmacokinetics study of Genistein**

| Pharmacokinetics parameter       | Predicted remarks | Unit         |
|----------------------------------|-------------------|--------------|
| Drug likeness                    |                   |              |
| Lipinski                         | Yes               | Yes/No       |
| Ghose                            | Yes               | Yes/No       |
| Egan                             | Yes               | Yes/No       |
| Veber                            | Yes               | Yes/No       |
| Muegge                           | Yes               | Yes/No       |
| Bioavailability Score            | 0.55              | N/A          |
| Absorption                       |                   |              |
| Water solubility                 | -3.595            | log mol/L    |
| Caco2 permeability               | 0.9               | log Papp in 10-6 cm/s |
| Human intestinal absorption      | 93.387            | % Absorbed   |
| Skin Permeability                | -2.735            | log Kp       |
| P-glycoprotein substrate         | Yes               | Yes/No       |
| P-glycoprotein inhibitor         | No                | Yes/No       |
| Distribution                     |                   |              |
| VDss (human)                     | 0.094             | log L/kg     |
| BBB permeability                 | -0.71             | logBB        |
| CNS permeability                 | -2.048            | logPS        |
| Metabolism                       |                   |              |
| CYP1A2 inhibitor                 | Yes               | Yes/No       |
| CYP2C19 inhibitor                | Yes               | Yes/No       |
| CYP2C9 inhibitor                 | Yes               | Yes/No       |
| CYP2D6 inhibitor                 | No                | Yes/No       |
| CYP3A4 inhibitor                 | Yes               | Yes/No       |
| Excretion                        |                   |              |
| Total clearance                  | 0.151             | log ml/min/kg|
| Toxicity                         |                   |              |
| Eye corrosion                    | No                | Yes/No       |
| Hepa-toxicity                    | Yes               | Yes/No       |
| Acute oral toxicity              | 1.842             | Kg/mol       |
| OATP2B1 inhibitor                | Yes               | Yes/No       |
| OATP1B3 inhibitor                | No                | Yes/No       |
| OCT2 inhibitor                   | No                | Yes/No       |
| AMES toxicity                    | No                | Yes/No       |
| hERG I inhibitor                 | No                | Yes/No       |
| Carcinogenicity                  | No                | Yes/No       |
| BSEP inhibitors                  | No                | Yes/No       |
| Anti-cancer affects              |                   |              |
| P-GP Inhibitor                   | No                | Yes/No       |
| Aromatase                        | Yes               | Yes/No       |
| ER binding                       | Yes               | Yes/No       |
7. Concluding remarks:

Nowadays, breast cancer is the major leading cause of death due to have diverse pernicious side effects of proposed synthetic drugs, multi drug resistance issues and many more. But natural food products are given more attention to the people in combating diseases such as cancer insurgencies, mainly, breast cancer. For this reason, this current investigation was conducted to explore the better pharmacological activity of natural phytochemicals namely-Genistein that offer therapeutic activities in tissues of breast cancer patients, cell specific anti-breast cancer mechanism, and most importantly, possess potential synergistic activity with multidrug-resistant tumors. Here, the safety assessment of the Genistein conducted by the online-based server and predict their pharmacokinetics profiling as a safe therapeutic compound but more wet-lab based research is badly needed to discover the cell-specific breast cancer controlling, toxicological mechanism and their pharmacokinetics properties.

Author’s contribution:

M.S., P.B., and D.D. have equally contributed for the conceptualization and study designing; M.S., P.B., M.A.A., D.D., H.S., S.A., M.S.K., M.A.H., P.P., and N.I. equally participated in data collection, writing, and draft preparation; M.A.R., and A.A.M. review and editing; Visualization
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