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Insilico studies of daidzein and genistein with human estrogen receptor α

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ABSTRACT

Objective: To evaluate potential use of daidzein and genistein in the prevention of breast cancer. **Methods:** Molecular docking of human estrogen receptor α (HER α) with daidzein and genistein were done by using Discover studio 3.1. Various analogs of daidzein and genistein were docked with HER α to enhance the binding affinity and locate the pharmacophoric groups. **Results:** Replacement of pharmacophoric group with methylsulphonmide (–NH–SO2–CH3) in daidzein and cyanide (–CN) in genistein were found to be sterically more compatible with significant increased energy values and could serve as probable lead molecules. The docking sore (E–value) obtained for genistein and daidzein was –217.67 and –220.63 respectively which shows their high affinity for HER α. **Conclusions:** Lower affinity and the side effects associated with conventional drugs needs an improved solution. The present findings showed that daidzein and genistein can play a potent role in breast cancer prevention.

1. Introduction

Soy food intake is associated with longer survival and low recurrence among breast cancer patients. Several epidemiological studies correlated high dose consumptions of soy Isoflavones [1] with multiple beneficial effects on breast and prostate cancers, menopausal symptoms, osteoporosis atherosclerosis and stroke, and neurodegeneration [2, 3]. In addition to the amount of soy isoflavones consumed, the form, food source, timing of isoflavone exposure, estrogen receptor status of tumors, and equol–producer status and hormonal profile of individuals may modify the association between soy isoflavone intake and the risk of breast cancer [4]. Compared to the US, low age standardized and age specific breast cancer incidence rates were found in Kurdish women and variations have been observed in the level of expression of ER in the rural and urban women [5, 6].

Although relatively limited research has been conducted, and the clinical trials often involved small numbers of subjects, but there is no evidence that isoflavone intake increases breast tissue density in pre– or postmenopausal women or increases breast cell proliferation in postmenopausal women with or without a history of breast cancer [7]. A large, population–based, prospective cohort study provides strong evidence of a protective effect of soy food intake against premenopausal breast cancer [8].

Breast cancer is the second leading cause of cancer in the western countries compared to the Asian countries, and about 60% percent of breast cancers are detected as estrogen receptor alpha positive (ER α) cancers. ER α is essential for mammary gland development and plays a central role in breast cancer development, but ER α can mediate estrogen–induced cell proliferation in an autocrine mode in ER α positive breast cancer cell lines [9]. The diagnostic strategies with the highest positive predictive value (88%) included hormone receptors (estrogen or progesterone) and mammaglobin in serial manner [10].

The present study was focused in silico analysis of the isoflavones (daidzein and genistein) to evaluate their potential in the prevention of breast cancer. Molecular docking was done by using Human estrogen receptor alpha (HER α). Closely interacting amino acids in the ligand binding cleft were identified using Discovery studio 3.1. Various analogs of daidzein and genistein were docked with the HER α to further enhance the binding affinity and locate the pharmacophoric group which allows developing certain novel molecules thereby serve as lead for the drug development.

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2. Materials and methods

2.1. Computational methodology

Present study was confined to estrogen receptor alpha (IA52), isoflavones (i.e. daidzein and genistein) and their analogs to determine the binding affinity with Estrogen α and the detailed study of their interaction pattern by using various molecular docking and Receptor-ligand interaction tools.

2.2. Retrival of atomic data

The X-ray crystallographic structure of the Human estrogen receptor α (alpha) was retrieved from the Protein data bank. The Accession no. of the PDB estrogen receptor retrieved from the PDB was IA52 (Figure 1). ER α is composed of 595 amino acids, whereas ER β is 530 amino acids. The N-terminal A/B domain (also known as the AF-1 domain) has a ligand independent transactivation function and has 17% amino acid homology between the ERs. The highly conserved central C region, with 96% homology, encompasses the DNA-binding domain (DBD). The flexible hinge, or D region, contains nuclear localization signal (NLS) information and links the C-domain to the multifunctional C-terminal E/F domain. The E/F domain (also known as the AF-2 domain) of the ERs shares 53% amino acid homology and contains the ligand binding domain (LBD) and the ligand-dependent transactivation domain, including the cofactor-binding groove to which cofactors are recruited when the ER becomes activated. For ER α, it has been shown that interaction between the alpha transactivation function (AF) domains is essential for effective transactivation [11, 12].

Structure for daidzein (CID 5280961; IUPAC Name: 5, 7-dihydroxy-3-(4-hydroxyphenyl) chromen-4-one) and genistein (CID 5281708; IUPAC Name: 7-hydroxy-3-(4-hydroxyphenyl) chromen-4-one) were retrieved from NCBI Pubchem compound. The following retrieved structures were validated and all the heteroatoms were removed for efficient molecular docking studies.

2.3. Molecular dynamics simulation

HER α retrieved from the protein data bank was used for the detailed study of its structure and the binding pattern with ligands i.e. isoflavones and their analogs to exactly find the binding pattern of ligands in the binding site and to locate the pharmacophoric part of the ligands for their modification to enhance the binding affinity. Ramachandran plot was made using Discovery studio platform to determine the presence of various secondary structures in the 3D structures of ER α. The docking analysis of daidzein and genistein (Figure 2) with Human Estrogen receptor was carried out by HEX docking software. It explores ways in which two molecules, such as drugs and a receptor fit together and docks to each other.

Hex is an Interactive Molecular Graphics Program for calculating and displaying feasible docking modes of pairs of protein and DNA molecules. Hex can also calculate Protein-Ligand Docking, assuming the ligand is rigid, and it can superpose pairs of molecules using only knowledge of their 3D shapes. It uses Spherical Polar Fourier (SPF) correlations to accelerate the calculations and its one of the few docking programs which has built in graphics to view the result.

The parameters used for the docking process via HEX docking software were:

- Correlation type – Shape only
- FFT Mode – 3D fast life
- Grid Dimension – 0.6
- Receptor range – 180
- Ligand Range – 180
- Twist range – 360
- Distance Range – 40

2.4. Pharmacophore identification

The identification of closely interacting functional group of the ligand with the receptor was done by using Ras Mol visualization tool. Genistein (4′, 5,7-trihydroxyisoflavone), the predominant isoflavones in the plant family Leguminosae, which includes the soybean, may be helpful in preventing and treating several types of cancers, principally breast and prostate cancers [13]. Docked structure was further analyzed with the RasMol and Discovery Studio to exactly locate the pharmacophoric part of the ligand (Figure 3). The active functional group found to be interacting directly and acting as pharmacophore were identified as hydroxyl group (-OH) and Carbonyl group (-CO).

2.5. Analog preparation

Various novel molecules were developed using Marvin Sketch after identification of the probable pharmacophoric group and docking was done to find the best suitable ligand with high affinity. The modifications at the probable binding sites were done using Marvine Sketch platform following the Silverman′s "The organic chemistry of drug design and drug action"and "Lipinski′s rule of five" (Figures 4 and 5) [14, 15].

The side effects of these drugs make the need for the necessity of new improved drugs hence in our research study we focused our work on the major isoflavones from legumes to find out their estrogenic potential and also to find the suitable analogues of them with high binding affinity, which could be a possible lead molecule. Modifications were done at the probable pharmacophoric sites which were identified as hydroxyl (¯OH) and carbonyl (¯CO) group after docking analysis using Hex and RasMol software.

3. Results

3.1. Protein preparation and Ramachandran plot of HER α

Ramachandran plot (Figure 6) shows the conformations of the ψ and φ angles which are possible for an amino-acid residue in a protein and it was made by using Discovery studio3.1. For HER α it showed the prevalence of the right

handed α helix and parallel β sheets.

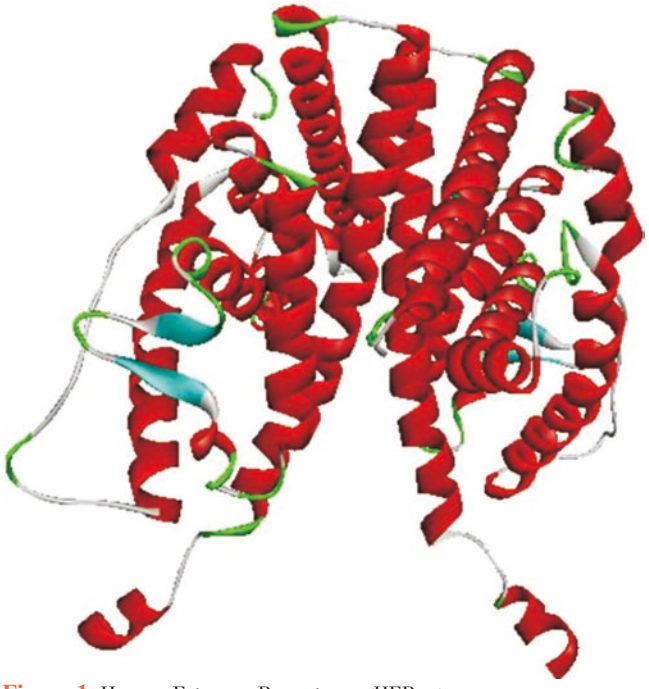

Figure 1. Human Estrogen Receptor α (HER α).

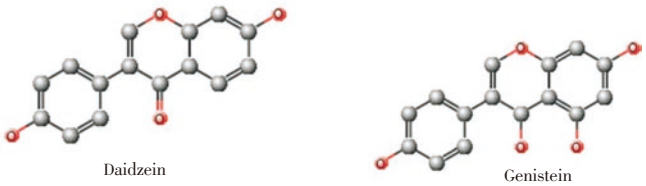

Figure 2. Daidzein and Genistein.

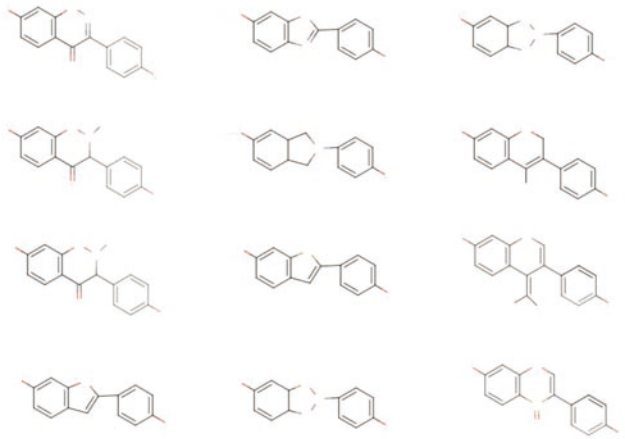

Figure 3. Pharmacophoric part of daidzein.

3.2. Determination of the binding affinity of isoflavones with HER α

We focused to evaluate the phytoestrogenic property of the isoflavones from the legumes which indirectly show the potential their potential in the prevention of the hormone related cancers. The molecules binding to a receptor, inhibit its function, and thus act as drug [16]. Estrogen receptor α (IA52) with two chains *i.e.* chain A and chain B retrieved

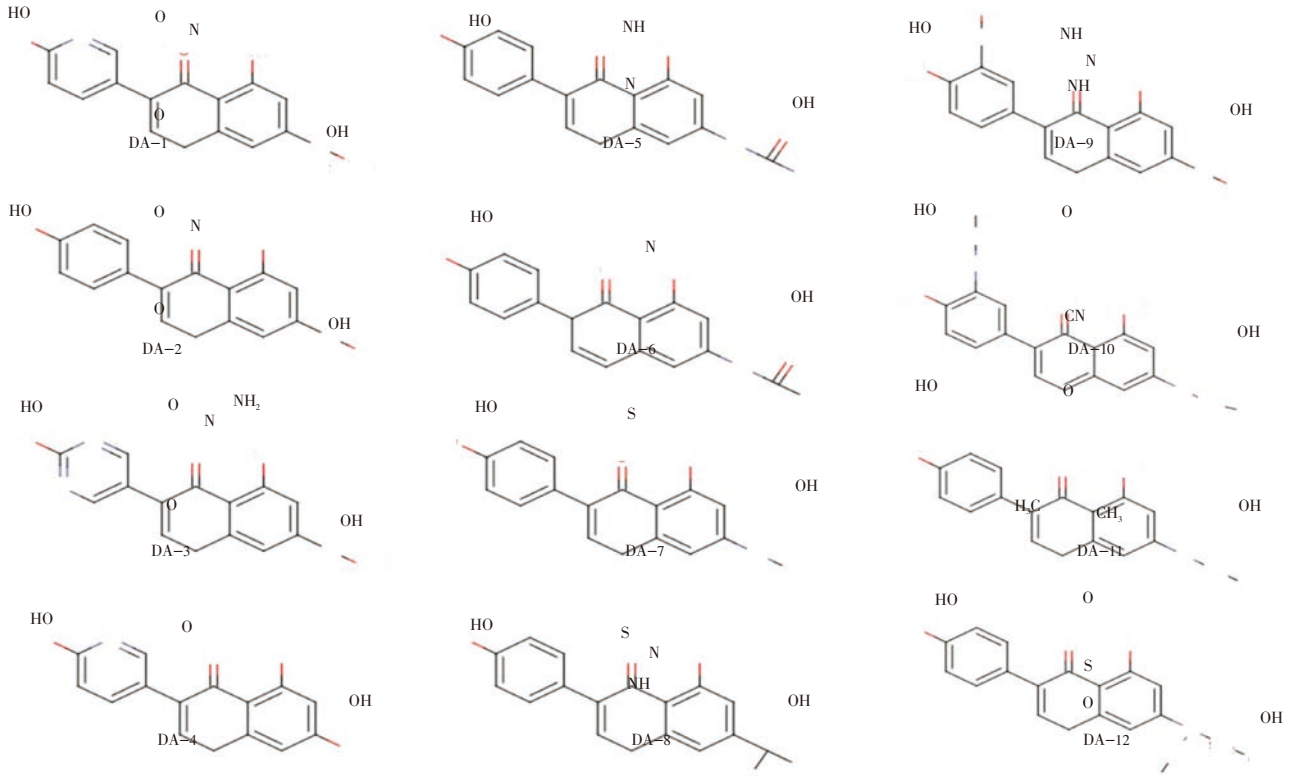

Figure 4. Daidzein analogs.

from the PDB was docked with daidzein and genistein (retrieved from Pubchem) which are the major isoflavones in the legumes. Both ligands were found to be exhibiting high binding affinity for the estrogen receptor with docking score of −217.61 and −220.63 respectively (Figure 7).

3.3. Drug like novel molecules

Most commonly used anti breast cancer drugs *i.e.* Tamaxifen, Toremifene and Ralaxofene are targeted to the estrogen receptor, and Raloxifene and tomerifene on docking produced the energy value of −158.37 and −108.0 respectively [17]. The net benefit of tamoxifen reduces with increasing age because of a high risk of stroke in older women. Older Korean women have more risk than benefit from tamoxifen chemoprevention [18]. The best novel molecules with high binding affinity were identified based on the docking score (Figures 8 and 9). Analog with methylsulphonmide (−NH−

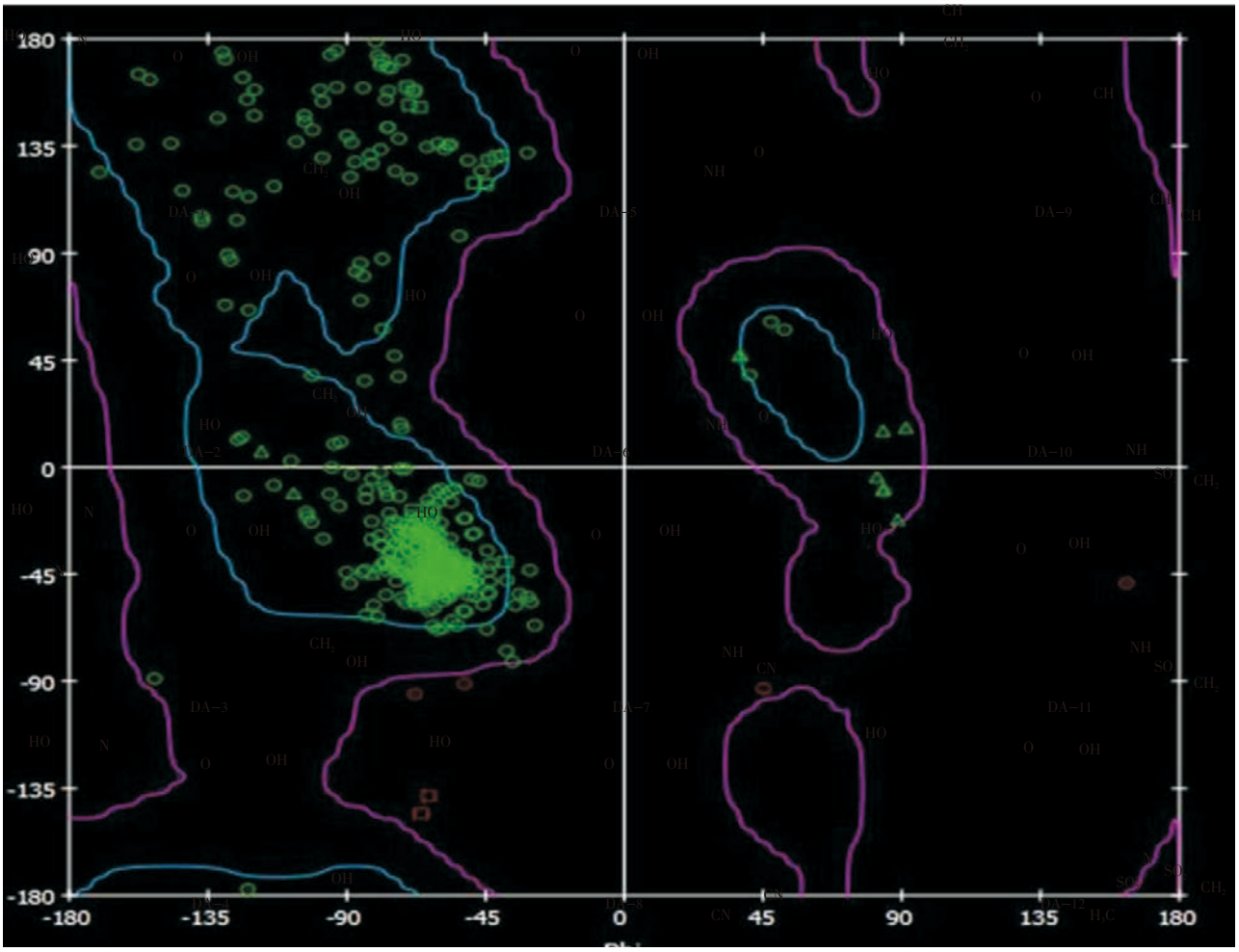

Figure 5. Genistein analogs.

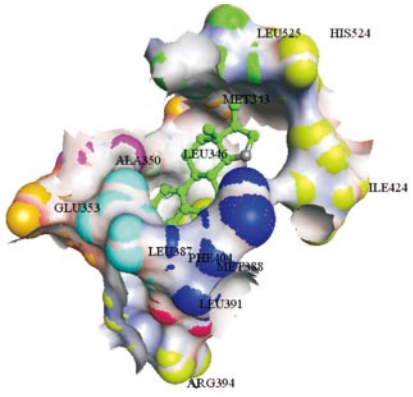

Figure 6. Ramachandran plot for HER α .

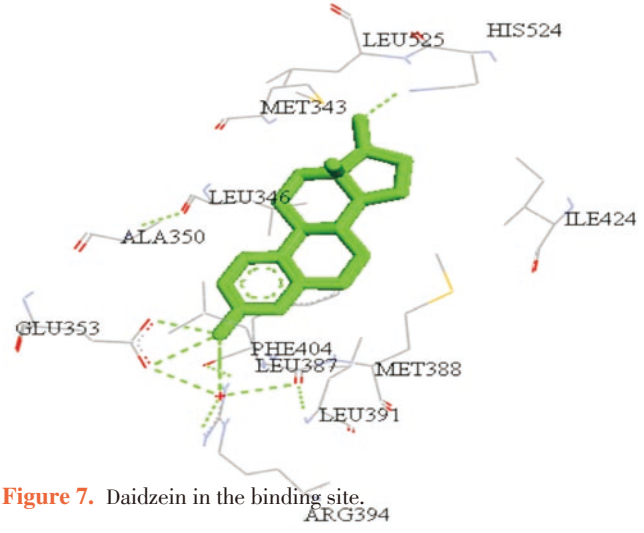

Figure 7. Daidzein in the binding site.

SO_2–CH_3) group and cyanide group (–CN) were found to be sterically more compatible.

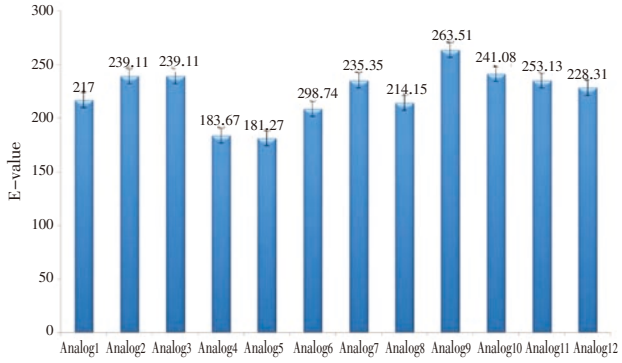

Figure 8. E value of Daidzein Analogs.

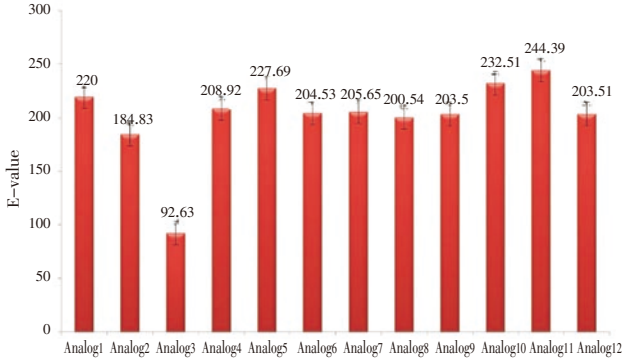

Figure 9. E value of Genistein Analogs.

For genistein, analogs 10 and 11 showed the lowest energy value and it was found to be –263.51 and 241.08 respectively. And for daidzein, analog 11 and 12 showed the highest energy value. Thus after docking studies of the daidzein and genistein it was possible to design more potent analogs with higher energy values which could be better lead molecule for the drug development.

3.4. Ligand binding site

Ligand binding site of estrogen receptor α was located by Discovery studio 3.1 and closely interacting amino acids in the binding site were identified as F339 (Phenylalanine), E 388 (Glutamic acid), L327 (Leucine), R329 (Arginine), H459 (Histidine), L 460 (Leucine) (Figure 10).

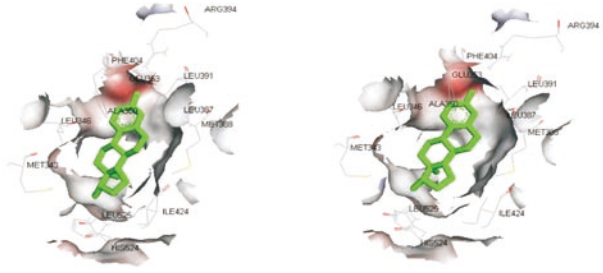

Figure 10. Ligand binding site.

4. Discussion

Estradiol plays an important role in the progression of breast cancer, and the biological effects of estrogen are mediated by its binding to the HER α and Hydroxylation of estrogens may modulate postmenopausal breast cancer risk [19, 20]. The estrogen receptor alpha (ER α) is responsible for controlling transcription of nuclear DNA necessary for human development and is considered to be an important component of breast cancer signaling network and is emerging as a novel biomarker of the disease. Selective estrogen receptor modulators (SERMs) are synthetic compounds which are used to modulate ER activity. Different SERMs display varying combinations of agonistic, antagonistic and neutral effects upon estrogen receptors and being employed to treat a range of ER–related disorders. A common feature shared by many SERMs is the close arrangement of three aromatic rings similar to TPP cations [21, 22].

High dietary intake of soy isoflavones was found to be associated with lower risk of recurrence among postmenopausal patients with breast cancer positive for estrogen and progesterone receptor and those who were receiving anastrozole as endocrine therapy [23, 24]. Genistein exhibited a promising safety profile with positive effects on bone formation in a cohort of osteopenic, postmenopausal women and is also effective in ameliorating cardiovascular profiles in an experimental model of postmenopausal metabolic syndrome [25, 26]. The E–value for daidzein and genistein is comparatively higher than certain conventional drugs which are in common use for the breast cancer therapy i.e. tamoxifen (–49.0), raloxifene (–158.0), toremifene (–108.0) [27].

Consumption of soy isoflavone is inversely associated with the risk of breast cancer [28]. A recent epidemiologic study involving 1954 female survivors of breast cancer also suggested a lower risk of breast cancer recurrence with high intake of daidzein and glycetin among both postmenopausal women and tamoxifen users [29]. Daily supplementation for 2 y with 80–120 mg soy hypocotyl isoflavones has minimal risk in healthy menopausal women and about 33% decrease in mammary tumor growth has been observed by genistein in rats [30, 31]. Reduced bone loss has been observed by D Lee Alekel *et al.*[32] in case of post–menopausal women with high soy isoflavones consumption and after intake of soy milk or other soy supplements, isoflavones reach exposure levels in breast tissue at which potential health effects may occur [33].

Genistein derivatives exerted their effects at concentrations 10–15 times lower than the parent compound, decreasing the likelihood of significant ER α pathway activation, which has been a concern for genistein. Hence, these compounds might play a useful role in breast cancer chemoprevention [34]. Clinical studies in osteopenic and osteoporotic, postmenopausal women support the breast and uterine safety of purified naturally derived genistein administered for up to 3 years [35]. Also it has been suggested that the clinical effectiveness of isoflavones might partly depend on the ability to produce equol, a gut bacterial metabolite of daidzein showing stronger estrogenic activity than the predominant Isoflavones [36]. Considering adverse

effects, TMX was 6 times more adverse effects and there is an immense need to develop drugs that will decrease the incidence of estrogen receptor-negative breast cancer in women at high risk of developing the disease [37, 38]. For postmenopausal women without a uterus, the benefit/risk ratio is similar [39].

Interaction pattern of a drug to a particular receptor play very important role for the execution of its pharmacological effects. Molecular docking provides the best platform for the protein ligand interaction. The results suggest that increased consumption of isoflavones could provide a way to control the occurrence of the breast cancer in peri and postmenopausal women.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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