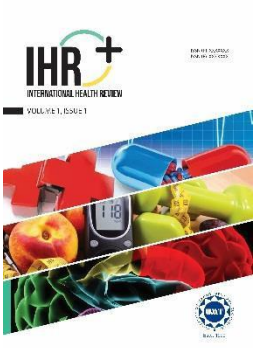

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Article: ***In Silico* Study of the Inhibitory Effect of Isoflavones on the Genes Frequently Reported in the Pathophysiology of Breast Cancer**

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In Silico Study of the Inhibitory Effect of Isoflavones on the Genes Frequently Reported in the Pathophysiology of Breast Cancer

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Abstract

Phytoestrogens are naturally occurring plant-derived active substances found in a wide variety of foods. Numerous health benefits of phytoestrogens include the reduced occurrence of breast tumors. Overexpression of the protein serine-hydroxyl-methyl-transferase-2 (SHMT2) has been reported in breast cancer. In one-carbon metabolism SHMT2 enzyme is present, which is necessary for cancer cell proliferation in a low glucose environment. Hence, this study focused on natural isoflavones that have a toxic effect on the development of breast cancer cells without harming normal cells. The structure of the protein SHMT2 was retrieved from the protein data bank and the structure of isoflavones was determined using the PubChem database. The docking of isoflavones with SHMT2 was done to determine their inhibitory effect on the latter since it causes the rapid development of breast cancer cells. It was identified that two isoflavones (6-O-acetyldaidzin and malonyldaidzin) have a high affinity to bind with the protein because of hydrogen bonding. This study highly recommends conducting clinical trials on human beings based on the interaction between phytochemicals and SHMT2 gene to determine the safe dosage level of the former.

Keywords: breast cancer, docking, isoflavones, phytoestrogens, serine hydroxyl-methyl-trans-ferase-2 (SHMT2) protein

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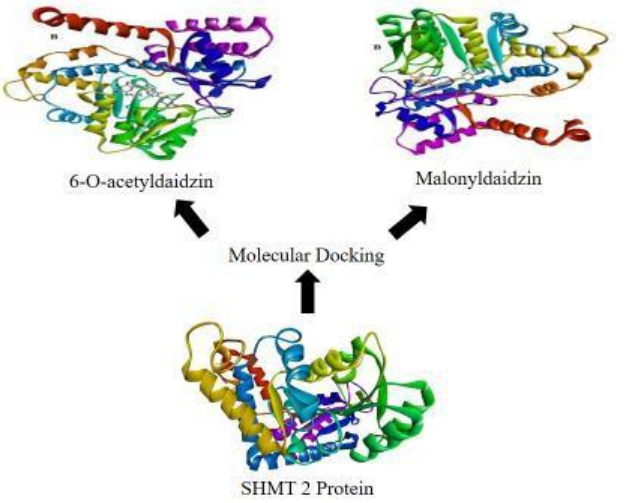
Graphical Abstract



Highlights

- Different isoflavonoids bound with SHMT2 protein and showed the possibility of the inhibition of SHMT2 which is responsible for breast cancer progression

Introduction

Phytoestrogens are naturally occurring plant-derived active substances found in a wide variety of foods and display an estrogen-like biological activity [1]. There are favorable health benefits of soy isoflavones that include the reduced occurrence of cardiovascular diseases (CVD), breast tumors, and menopausal symptoms [2]. The prevalence of breast cancer is higher in the Asian population as compared to the rest of the world [3]. Notable examples of phytoestrogens occurring in plants and seeds include isoflavones, coumestans, and lignans which exhibit various cytotoxic effects on health [4]. Notable sources of isoflavones are soy and soy products, milk and milk products, eggs, legumes, cheese, tofu, clover, beans, lentils, and seafood [1]. Recent studies have reported consistently that the frequent intake of a diet high in phytoestrogens is linked with decreasing chances of CVD as well as prostate and breast cancer [5].

Among American women, breast cancer is the most common cancer and 1 out of every 33 women is diagnosed with it in her lifetime [6]. Moreover, the prevalence of breast tumors in women is considered the second leading cause of death after lung cancer. Several protein regulations have been recorded in breast cancer, such as an overexpression of serine-hydroxyl-methyl-transferase-2 (SHMT2) protein [7, 8, 9]. SHMT2 enzyme participates in one-carbon metabolism, which is necessary for cancer cell proliferation in a low glucose environment [10, 11]. This is also supported by the results of a study in which SHMT2 downregulation led to the suppression of tumorigenesis in human hepatocellular carcinoma in response to soy phytoestrogens [12]. Phenolic compounds such as flavonoids have bioactive components and influence various biological activities. Citrus-derived naringenin isoflavones perform an anti-inflammatory role and they can be used in different drug formulations [13]. Epidemiological and *in vivo* studies recommend that the intake of a diet rich in isoflavonoids reduces the risk of breast cancer. According to their chemical composition, isoflavones are further classified into 4 types [1]: a) aglycones b) glycoside c) malonyl glucoside (malonyldaidzin, malonlygenistin, malonyl glycitein) d) acetyl glucoside (6-O-acetyldaidzin, acetylgenistin, acetylglycitein). To the best of our knowledge no *in silico* study has been conducted on the interaction of various phytoestrogens with SHMT2. The current research work aims to evaluate the hypothesis that various phytoestrogens bind with SHMT2. The outcome of the current study would furnish evidence for the said hypothesis, so that chemo-preventive trials can be pursued in the future.

Materials and Method

Protein Preparation

RCSB protein data bank was utilized for downloading 46.06 kDa *Bacillus stearothermophilus* ‘SHMT2’ protein with the sequence length 405 (PDB ID: 1KKP).

Ligand Preparation

PubChem (https://pubchem.ncbi.nlm.nih.gov/) was used to collect chemical information regarding various ligands includng identifiers such as SMILES, a mcule ID, CAS Number, InChI, IUPAC name, and InChIKey. The following ligands were evaluated: daidzein (ZQSIJRDFPHDXIC-

UHFFFAOYSA-N), genistin (InChI=1S/C21H20O10/c22-7-15-18(26)19(27)20(28)21(31-15)30-11-5-13(24)16-14(6-11)29-8-12(17(16)25)9-1-3-10(23)4-2-9/h1-6,8,15,18-24,26-28H,7H2/t15-,18-,19+,20-,21-/m1/s1), glycitein (DXYUAIFZCFRPTH-UHFFFAOYSA-N), 6-O-acetyldaidzin (ZMOZJTDOTOZVRT-DODNOZFWSA-N) and malonyldaidzin (MTXMHWSVSZKYBT-ASDZUOGYSA-N).

Sequence Information of Serine Hydroxyl-Methyl-Trans-Ferase-2 (SHMT2) Protein

The sequence of this molecule was generated using the discovery studio client 2020 software (https://www.3dsbiovia.com/).

Docking Studies Using Mcule

Ligand information (identifier) was placed in the mcule docking online server and refined in drawer (https://mcule.com). The mcule system generated the 2D structure of a ligand which was used to target / dock the protein of interest. Alternatively, this system provided the opportunity to manually draw a ligand. After docking, the online system provided a program database (pdb) file of the ligand-protein complex. In this study, ligands comprise different phytochemicals and the target molecule is a protein (SHMT2). After docking by mcule, pdb files were generated with different docking scores (pose). Poses with high negative values were selected and downloaded (pdb file).

Stability, Bonding and Amino Acid Information of the Ligand

The retrived pdb files were processed and the following information was collected: the bindings between the ligands and the protein (SHMT2), the 2D structure of the ligand-protein complexes, particular amino acid residues of SHMT2 protein bonded with a specific ligand / a phytochemical, and the nature and location of the bond protein pocket / cavity.

Results

Structure of Target Protein

RCSB protein data bank was utilized for downloading 46.06 kDa Bacillus stearothermophilus 'SHMT2' protein with the sequence length 405 (PDB ID: 1KKP), determined by X-rays diffraction at the resolution of 1.93A^{o}. Figure 1 shows the crystalline structure of SHMT2 protein. This bacterial SHMT2 is closely related (isoforms) to the human SHMT2 protein.

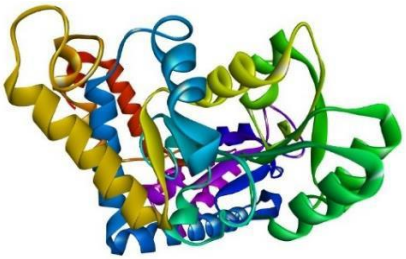


Figure 1. Crystal Structure of SHMT2

Analysis of the Interaction between SHMT2 and Isoflavones

SHMT2 offers a window of opportunity for intervention because of its role in cancer progression. Isoflavones found in different foods inhibit the function of SHMT2 proteins, thus their usage serves as a cost-effective intervention strategy. The results of the docking of SHMT2 with various flavonoids are described in Table 1.

Table 1. Docking Result of SHMT2 with Various Flavonoids

Types of Isoflavones	Docking Score	Bond
Daidzein	-7.6	3 Hydrogen 2 pi-pi stacked 1 pi-alkyl 2 pi-cation bond
Genistein	-7.5	3 hydrogen 1 pi-pi stacked 3 Carbon hydrogen 1 pi-cation
Glycitein	-6.7	1 hydrogen 2 carbon-hydrogen bond 3 pi-alkyl
6-O-acetyldaidzin	-8.4	5 hydrogen 1 pi-pi stacked 3 carbon-hydrogen
Malonyldaidzin	-6.7	4 hydrogen 1 pi-pi stacked 4 carbon-hydrogen 2 pi-cation

Docking Analysis

Among the evaluated compounds, daidzein and genistein formed eight bonds with SHMT2, indicating that phytochemicals should be encouraged to forestall the activation of cancer-causing proteins. The interaction of glycitein with SHMT2 protein showed the formation of six bonds, demonstrating the linkage between the ligand and the protein but not to the extent observed in the previous two cases. The docking of isoflavones with 6-O-acetyldaidzin showed nine different types of bonds. Interestingly, the binding of malonyldaidzin with SHMT2 protein revealed eleven bonds, thus surpassing all evaluated compounds. Hence, there is a huge potential for using this compound as a promising isoflavone for the inhibition of SHMT2 protein. The docking of daidzein, genistein, glycitein, acetyldaidzin, and malonyldaidzin with SHMT2 protein is described in figures 2, 3, 4, and 5, respectively.

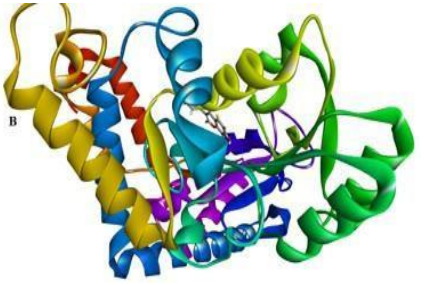


Figure 2. Docking of Daidzein with SHMT2

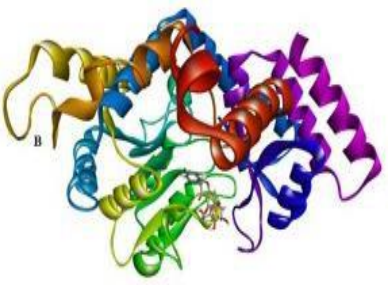


Figure 3. Docking of Genistein with SHMT2

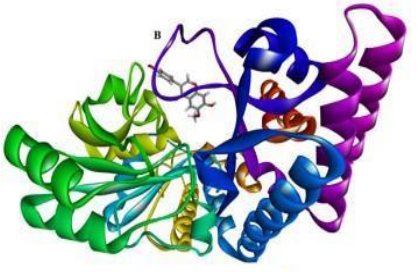


Figure 4. Docking of Glycitein with SHMT2

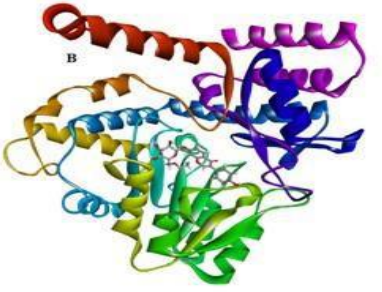


Figure 5. Docking of 6-O-acetyldaidzin with SHMT2

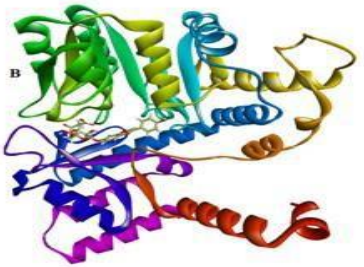


Figure 6. Docking of Malonyldaidzin with SHMT2

Discussion

Different types of bonding between various isoflavones and SHMT2 includes hydrogen bonding, pi-pi stacked, pi-alkyl, and pi-cation bonds. The bonding between daidzein and SHMT2 (demonstrated in Figure 2) shows the formation of three hydrogens, three pi-pi stacked, one pi-alkyl, and three pi-cation bonds, thus revealing the stability of the complex. The bonding between glycitein and SHMT2 protein (shown in Figure 3) includes one hydrogen, two pi-pi stacked and pi-alkyl bonds, which were also reported by other researchers [14]. These specific bonds provide isoflavones with their anti-proliferative and antioxidant properties. The bonding of 6-O-acetyldaidzin and malonyldaidzin with SHMT2 protein shows that these isoflavones can be used to inhibit the particular protein due to the presence of six hydrogen, one pi-pi stacked, two-carbon hydrogen, and a pi-alkyl bond. Numerous studies have reported the beneficial effect of isoflavones malonyldaidzin in inhibiting the mammary tumor cells lines [15, 16]. This research shows that 6-O-acetyldaidzin and malonyldaidzin are two isoflavones that fulfil all the properties of a biologically active molecule due to the presence of more hydrogen bonds than any other phytochemicals and can be used as active drug components during human clinical trials. The results of this *in silico* study are in line with another research which supports that these phytochemicals work against the progression of breast tumor cells by inhibiting the SHMT2 enzyme [17]. Soy products contain a reasonable amount of isoflavones that have estrogenic properties and they also reduce the risk of breast cancer. Aglycones isoflavones (daidzein and genistein), primarily found in soybeans, have the metabolic characteristics that make them useful in the

chemo-prevention of both breast and prostate cancer and also in killing several carcinogens [18]. Docking between isoflavones and SHMT2 protein revealed that it is a potent inhibitor of estrogen receptors and expresses anti-cancer properties. Biological activities of these isoflavonoids (genistein) with a tamoxifen may induce antitumor immunity and increase the survival rate of cancer patients [19]. The cytotoxic effect of flavonoids interferes with the signal transduction pathway to limit cancer cell proliferation, angiogenesis and increases apoptosis [16, 20]. It also suggests that the isoflavones take part in the stimulation of different enzymes such as glutathione peroxidase to combat oxidative stress inside the body. The bioavailability of plant isoflavones used to produce the anticancer effect is limited, so their combination with a therapy is preferred to produce the synergistic results useful in reducing cancer cell division and increasing the rate of apoptosis [21].

Conclusion

SHMT2 is defined as a concern in most cases of breast cancer continuation and remains a possible target for therapeutic intervention. We observed the link of phytochemicals with SHMT2 in most cases of breast cancer by means of the docking method, hence it's prudent to analyze this technique through *in vitro* researches. This *in silico* examination may be used as a piece of evidence to support human scientific trials.

Declaration of Interest

The authors declare no potential conflict of interest. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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