Clinical Nutrition Experimanetal

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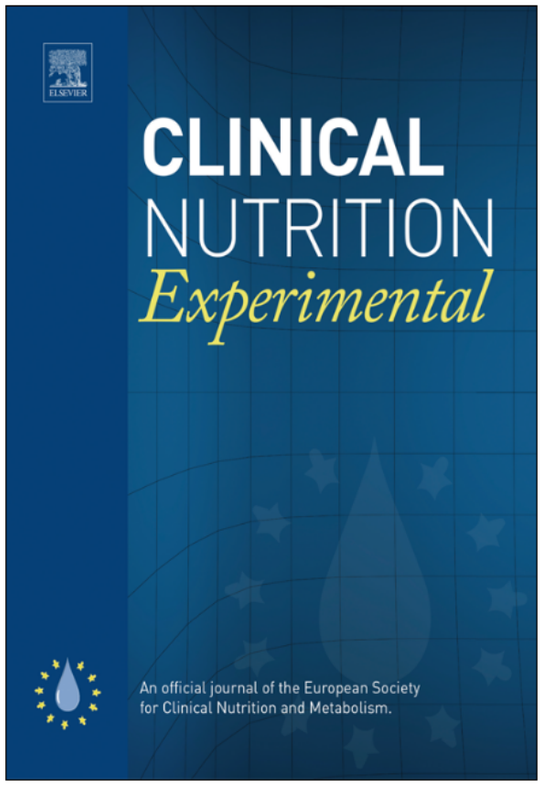
Submission date: 16-May-2023 01:44PM (UTC+0700)
Submission ID: 2094432273
File name: Clinical_Nutrition_Experimanetal.pdf (2.81M)
Word count: 3794
Character count: 21468



Clinical Nutrition Experimental 23 (2019) 15–22

Contents lists available at ScienceDirect

Clinical Nutrition Experimental

journal homepage: http://www.clinicalnutritionexperimental.com

Methodology

Search for aglycone isoflavone from soybean as candidate for pelvic organ prolapse treatment: *In silico* study of TGF-β1, Hsp70, and Bcl-xl signals

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ARTICLE INFO

Article history:
Received 5 April 2018
Accepted 27 November 2018
Available online 5 December 2018

Keywords:
Daidzein
Genistein
Glycitein
In silico

SUMMARY

The purpose of this study was to analyze molecular docking as a search for aglycone isoflavone in TGF-β, Hsp70, and apoptosis pathways that are useful for pelvic organ prolapse treatment. This *in silico* study involved procedures of searching for protein and nucleotide sequences, three-dimensional structure modeling, docking, and interaction analysis. Against TGF-β signal, the genistein facilitated interaction between TGF-β and TGF-β receptor, while against Hsp70 signal, the daidzein facilitated interaction of HSF and Hsp70. Against NF-κB signal for Bcl-xl gene, daidzein and glycitein facilitated interaction of NF-κB and Bcl-xl gene. It was concluded that aglycone isoflavone of the soybean could modulate TGF-β1, Hsp70, and Bcl-xl anti-apoptotic signals. Therefore,

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https://doi.org/10.1016/j.yclnex.2018.11.002

aglycone isoflavone derived from soybean can become an alternative nutrient or candidate for herbal product for pelvic organ prolapse treatment.

1. Introduction

Pelvic organ prolapse is a disease characterized by loss of pelvic wall support and uterine herniation or vaginal prolapse. Pelvic organ prolapse has some real impacts on life, including sexual dysfunction, social problem, depression, and unattractive body shape [1]. The number of people with this disease is expected to increase from 28.1 million in 2010 to 43.8 million in 2050 [2]. Although some phenomena are proposed to be the etiology of pelvic organ prolapse, this disease is multifactorial [3]. (see Figs. 1–3)

Transforming growth factor-β (TGF-β) is a multifunctional cytokine that regulates various cellular functions, including proliferation, apoptosis, and differentiation. TGF-β can stimulate fibroblast proliferation [4]. The role of TGF-β in pelvic organ prolapse is still unclear. In the case of pelvic organ prolapse, the expressions of mRNA and TGF-β1 protein are correlated negatively with the level of pelvic organ prolapse [5,6]. Other researcher found that TGF-β1 expressions in uterosacral ligament were almost similar between pelvic organ prolapse and the control [7]. In menopausal pelvic organ prolapse, there was a decrease in the fibroblast mitotic index compared with the pre-menopausal pelvic organ prolapse. After being stimulated by TGF-β, there was no difference in fibroblast proliferation response. This is presumably due to the downregulation of TGF-β receptor [8]. In the *in vitro* test using the strain model of extracellular matrix degradation, TGF-β1 application can inhibit the loss of extracellular matrix through TGF-β1/ Smad3 signals [6]. In addition, oxidative stress was involved in the TGF-β change in pelvic organ prolapse [9].

Hsp70 is an abundant protein and can be induced and expressed constitutively at normal growth temperature and serves as a chaperone molecule for the protein life cycle. Under stress condition, Hsp70 mRNA level will increase in 15 min. The search for active ingredient from plant was intended to induce HSP70 through phosphorylation in heat shock factor-1 (HSF-1) [10–12]. HSF is the HSP master regulator. When an organism experiences heat stimulus, inactive HSF-1 monomer will be converted into DNA-bound trimer that has transcriptional activity. Furthermore, HSF-1 trimer will bind to HSP gene promoter region, transcription begins and triggers upregulation of HSPs [13,14]. To date, involvement of Hsp70 in pelvic organ prolapse remains unclear. Previous study shown that in pelvic organ prolapse, there was an increase in the oxidative stress characterized by an increase in 8-oHdG and hydroxynonenal and decreased glutathione peroxidase compared with the control [5,15]. The researchers suggested that the increased stress was due to the inability of stress homeostasis by Hsp70. Increased oxidative status is associated with low basal level of Hsp70 [16].

Apoptosis, programmed cell death, is fundamental in a variety of physiological processes, including embryogenesis and tissue remodeling. Apoptosis occurs through intrinsic and extrinsic mechanisms. The extrinsic mechanism begins with the binding of the death-inducing ligand to the death receptor on cell surface. DNA damage, growth factor deprivation, and oxidative stress can induce intrinsic pathway. Initiation of the intrinsic pathway will trigger mitochondrial depolarization, thus releasing Cytochrome-c. The Cytochrome-c will release apoptotic protease activating factor-1 (APAF-1) and will form an apoptosome. The apoptosome will activate caspase-9, which then activates caspase-3 and induces the apoptosis [17–20]. In the pelvic organ prolapse, there are increases in mitochondrial apoptosis, apoptotic protein and decrease in the ratio of antiapoptotic protein compared with control [15,21]. Moreover, there are upregulations of proapoptotic protein, Cytochrome-c, caspase-3, and caspase-9 in the pelvic organ prolapse compared with control [22].

Of the various types of plants, soybean plant is considered as a source of protein long ago. Apart from high protein content, this plant also contains various nutritional and functional components,

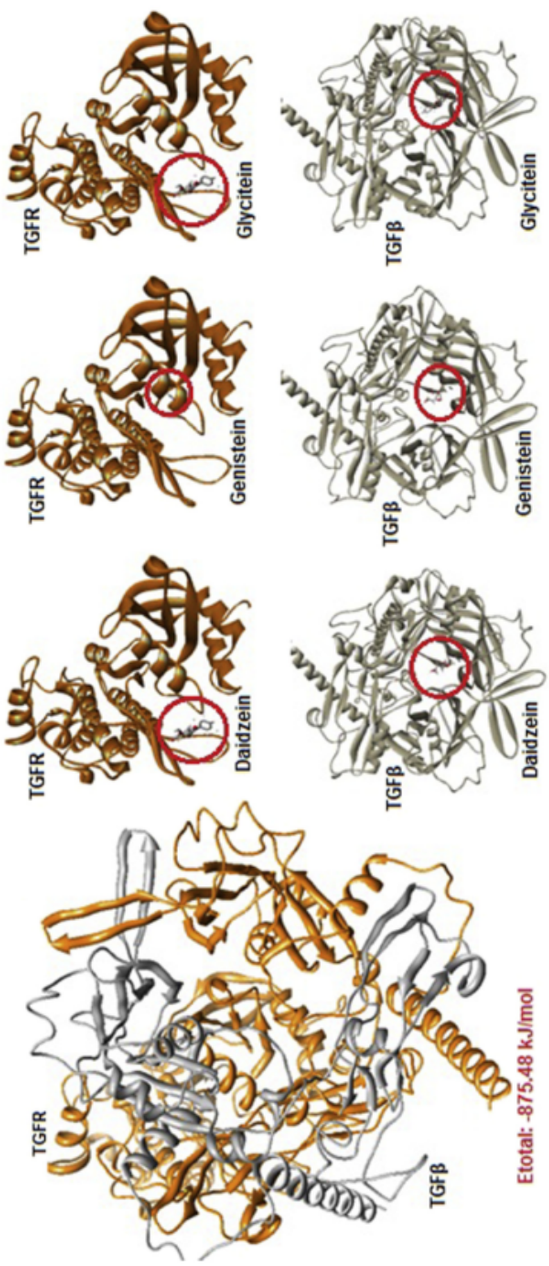

Fig. 1. The interaction between soy isoflavones with TGFβ1 and TGFR. The red circle indicates the site of interaction.

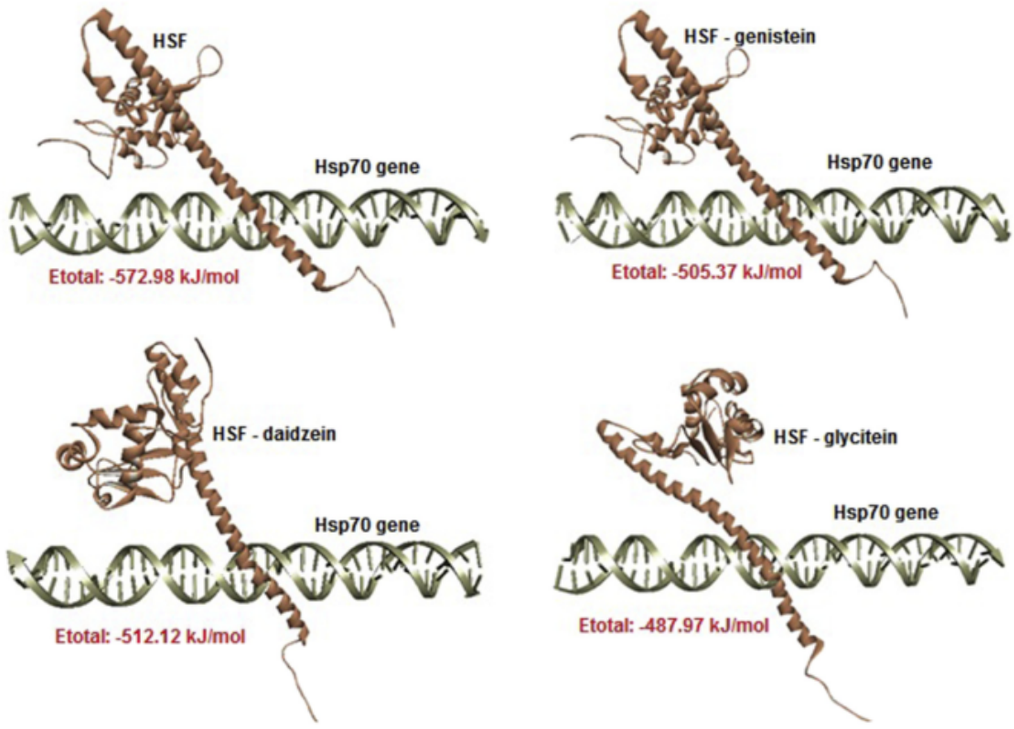

Fig. 2. Effect of soy isoflavone compounds on HSF interactions on the Hsp70 gene.

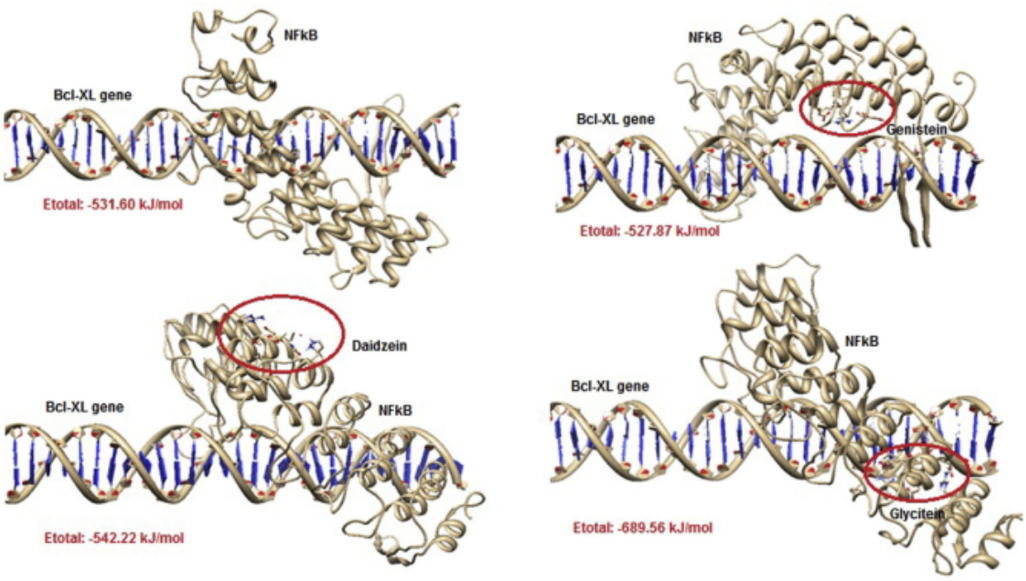

Fig. 3. Effect of soy isoflavone compound on NFκB interaction on Bcl-xl gene promoter.

including unsaturated fatty acid, fiber, mineral, and isoflavonoid [23]. Soybean contains four kinds of isoflavone structures, among others, aglycone (genistein, daidzein, and glycitein), glycoside (genistein, daidzein, and glycitein), acetyl glycoside (acetyl genistein, acetyl daidzein, and acetyl glycitein), and malonyl glycoside (malonyl genistein, malonyl daidzein, and malonyl glycitein) [24]. Biological activity of this compound in human body is determined by its chemical structure. Of the four structures, aglycone isoflavone seems to have beneficial effect on health compared with other structures [25]. Until now, to the knowledge of the researchers, there is no study investigating the benefits of aglycone isoflavone from soybean in pelvic organ prolapse treatment. Hence, the purpose of this study was to analyze molecular docking as an effort to search for aglycone isoflavone structure in TGF-β, Hsp70, and apoptosis pathways in pelvic organ prolapse.

2. Material and methods

2.1. Nucleotide sequence and protein structure retrieval

The structure of the active compound components of soybeans is obtained from PubChem Open Chemistry Database. Analyzed three active compounds, namely genistein (CID 5280961), daidzein (CID 5281708), and glycitein (CID 5317750). The sequence of TGF-β1 (GI: 11024652), TGFR (GI: 149020220), heat shock factor protein 1 (HSF) (GI: 274326531), Hsp70 gene sequence (GI: 24025636), NFκB (GI: 474298), and the Bcl xl gene (GI: 75992935), were obtained from the National Center for Biotechnology Information (NCBI) sequence database, the National Institutes of Health (NIH) (http://www.ncbi.nlm.nih.gov).

2.2. 3D structure modeling of bioactive components, proteins, and DNA

The 3D structure modeling of HSF, TGF-β1, TGFR, and NFκB is predicted using SWISS-MODEL webserver by homology modeling method [26,27]. The 3D structure of the protein is then validated by using Ramachandran plot analysis. 3D structure modeling of Hsp70 and Bcl-xl gene promoters is done with 3D-DART webserver. Convert *.sdf files into *.pdb files from active components of soybeans done using OpenBabel software [28].

2.3. Computational docking

Docking simulations were performed using HEX 8.0 software [29]. The docking protocol consists of three stages of visualization, namely rigid-body energy minimization, semi-flexible repair, and finishing refinement in an explicit solvent. After the execution of each stage, the docking confirmation is then scored and sorted by scoring function to facilitate the best conformation selection to be used at a later stage.

2.4. Inter-protein interaction analysis

The results of the next docking analysis will be visualized using Discovery Studio 4.1 software, LigPlot + and LigandScout 3.1 [30], while visualization and interaction analysis between proteins and DNA are performed using LUCPLOT software. Interaction analyzes are performed to look at the bonds that are formed, including hydrogen bonds, hydrophobic bonds, and van der waals bonds. Pharmacophore analysis was also performed to see the residues directly involved in the interaction process, as well as the minimization energy analysis to improve the structure and shape of the molecule during interaction.

3. Results

3.1. Genistein is thought to support TGF-β1 signaling activation

This analysis was conducted to see the possible effect of the active compound of soybean on TGF-β signaling. It was found that there is one possible soy isoflavone compound (ie genistein) to support the

interaction between TGF-β1 with its receptor, ie when genistein binds to TGFR before TGF-β1 binds (−897.01 kJ/mol). In another docking when genistein binds to TGF-β1 first before binding to TGFR, the required binding energy becomes larger (−835.54 kJ/mol). When daidzein and glycitein are present, the binding energy required for the interaction becomes greater, either when the compound binds to TGF-β1 before binding to TGFR, or when the compound binds to TGFR first before TGF-β1 binds.

3.2. Daidzein have a direct induction of Hsp70 gene transcription

To examine the possible role of soy active compounds in the induction of Hsp70 gene transcription, a docking analysis was performed between the Hsp70 transcription factor protein, HSF, with the promoter of the Hsp70 gene. This analysis shows that under normal conditions, the energy required by HSF to bind to the Hsp70 gene promoter is equal to −507.82 kJ/mol, with the bonds being formed as many as 18 (3 hydrogen bonds, 15 van der waals interactions). When simulated by interrupting daidzein in HSF protein before he binds to the Hsp70 gene promoter, it was found that the energy required to interact was lower than under basal conditions (−512.12 kJ/mol). In addition, it was found that the hydrogen bond, which is one of the strongest bonds, is reduced (hydrogen bonds are formed only, and the interaction of van der waals). In the simulations using genistein and glycitein compounds, the energy required by HSF to bind to the Hsp70 gene promoter shows that the required energy becomes slightly larger than under basal conditions.

3.3. Daidzein and glycitein induce transcriptional activation of anti-antiapoptotic gene Bcl-xl

Fig. 1 shows that the bonding site between glycitein and NFkB lies at the site where the bond between NFkB and the Bcl-xl gene, so it is suspected that this compound can support the induction of Bcl-xl gene transcription directly. This analysis was conducted to test the possibility of the ability of soy active compounds to support the transcription process of Bcl-xl anti-apoptotic gene. In the absence of the active compound, the energy required for the interaction is −531.60 kJ/mol (with 2 hydrogen bonds and 21 van der waals interactions). When daidzein is present, the binding energy becomes smaller (−542.22 kJ/mol); when there is genistein, energy bindings become larger (−527.87 kJ/mol); and when there is glycitein, the binding energy required for interaction between NFkB and the Bcl-xl gene promoter is much lower (−689.56 kJ/mol).

4. Discussion

TGF-β1 pathway is considered as the newest therapeutic target for pelvic organ prolapse [6]. As for TGF-β1 signal, firstly, TGF-β1 will interact with TGF-β type II (TGF-βII) receptor and then with TGF-β type I (TGF-βI) receptor. This interaction will trigger the rearrangement of heterotetrameric receptor complex. Next, there will be phosphorylation of TGF-βI, resulting in a bonding location for Smad2/Smad3 protein and leading to the phosphorylation. The phosphorylated Smad2/Smad3 protein will form heteromeric complex with Smad4 and moves to the nucleus to bind to the target promoter region of TGF-β gene. Expression of this gene is involved in the differentiation, proliferation, apoptosis, migration, and extracellular matrix development [31–33]. This study revealed that the interaction energy between TGF-β1 and TGF-β1 receptor was −875.48 kJ/mol. When there is genistein, the interaction energy becomes −897.01 kJ/mol. This indicates that genistein facilitates interaction between TGF-β1 and TGF-β receptors. On the other hand, daidzein and glycitein may make interaction of TGF-β1 and TGF-β receptors more difficult. Previous study shown that expression of TGF-β1 protein may change or is lower in prolapse case than the control without prolapse [34,35]. Thus, this study says that genistein is a candidate for pelvic organ prolapse treatment through modulation of TGF-β1 signals. Previous study demonstrated that genistein can increase mRNA expression of TGF-β1 and TGF-β1 protein [36,37].

Previous study stated that in pelvic organ prolapse, there was an increase in mitochondrial apoptosis compared with control [15]. Besides, the increased apoptotic protein and decreased ratio of anti-apoptotic protein to the apoptotic protein were also found [21]. Bcl-xl is an anti-apoptotic protein. In this study, daidzein and glycitein can facilitate the interaction between NFκB and Bcl-xl gene. This

indicates that these two compounds will support upregulation of anti-apoptotic protein in pelvic organ prolapse. Previous study indicated that daidzein can trigger upregulation of Bcl-xl [38], while there is no study that evaluate the effect of glycitein on Bcl-xl. This study also proved that daidzein can promote interaction between HSP70 gene and HSF. Further, this interaction will support HSP70 activity as an anti-apoptotic protein [39].

It is concluded that soybean-derived aglycone isoflavone can modulate TGF-β1, Hsp70, and Bcl-xl anti-apoptotic signals. Therefore, aglycone isoflavone sourced from soybean can become an alternative nutrient or candidate for herbal product for pelvic organ prolapse treatment.

Conflict of Interest

All authors state that there is no conflict of interest in the study or publication of this article.

Authors contribution

All authors have critically reviewed and approved the final version of the manuscript. PB, IWAW, IKO, DS conceived and designed the study, conducted research, provided research materials, and collected and organized data. PB analyzed and interpreted data. PB, IWAW, IKO, DS wrote initial and final draft of article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yclnex.2018.11.002.

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