

Molecular docking of genistein on estrogen receptors, promoter region of BCLX, caspase-3, Ki-67, cyclin D1, and telomere activity Tahun Terbit 2019

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Original Article

Molecular docking of genistein on estrogen receptors, promoter region of BCLX, caspase-3, Ki-67, cyclin D1, and telomere activity



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المخلص

أهداف البحث: تهدف هذه الدراسة للتحقيق في تشكيل مستقبلات هرمون الأستروجين بواسطة تركيب مضاد –الأستروجين ودور الجينيسيتين ضد تنظيم عملية النسخ من الجينات المشاركة في الانتشار، وموت الخلايا المبرمج ونشاط التيلومير.

طرق البحث: تم إجراء البحث باستخدام أسلوب سوليكو بحيث يكون الإرساء هو أهم طريقة تم تنفيذها بواسطة برمجيات الهيكل 8.0 وقاعدة البيانات هانوك. ثم تم عمل تحليل التفاعل لملاحظة التفاعلات بين الجينيسيتين وعدد من البروتينات والجينات ذات الصلة باستخدام برامج الاكتشاف.

النتائج: لم يظهر التفاعل بين مستقبلات هرمون الأستروجين – الفا مع الجينيسيتين تشكيل أي رابطة. وهكذا التفاعل، الذي يمكن حدوثه، لن يكون فاعلا لأنه ليس مستقرا. وعلى العكس، عندما يكون التفاعل مع مستقبلات هرمون الأستروجين- بيتا، اثنان من الروابط الهيدروجينية وأربع من الروابط الطاردة للماء، هيدروكلوريد تفاعل مع مستقبلات هرمون الأستروجين – الفا بواسطة اثنان من الروابط الهيدروجينية وثلاثة من الروابط الطاردة للماء. سيكون من السهل للمركب الحث على تنشيط النسخ للجينات المنروسة.

الاستنتاجات: إعطاء الجينيسيتين ممكن أن يزيد النشاط الجينومي لمركبات مستقبلات هرمون الأستروجين التي ترتبط بموت الخلايا المبرمج، والانتشار ونشاط التيلومير.

الكلمات المفتاحية: موت الخلايا المبرمج؛ مستقبل هرمون الأستروجين؛ جينيسيتين

Abstract

Objectives: This study aims to investigate the modulation of estrogen receptors by estrogen and the role of genistein in the transcriptional process that regulates genes involved in the proliferation, apoptosis, and telomere activity.

Methods: The research was conducted *in silico*, wherein docking, the most important method, was carried out using Hex 8.0 software and HADDOCK web server. Interaction analysis was subsequently done to observe the interactions between genistein and several related proteins and BCLX, Casp3, Ki-67, CyclinD1, hTERT, and POT1 genes using Discovery Studio, LigPlus, and NUCPLOT.

Results: The interaction between ER α with genistein was not found to form a single bond. Thus, the interaction that may occur will not be effective because it is not stable. Conversely, when interacting with ER β , two hydrogen bonds and four hydrophobic bonds, MPP dihydrochloride interacted with ER α via two hydrogen bonds and three hydrophobic bonds. The ER β /eNOS

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complex will be comparatively easier to induced by the transcriptional activation of BCLX, Casp3, Ki-67, CyclinD1, hTERT and POT1 genes.

Conclusions: Administration of genistein can increase the genomic activities of the estrogen-eNOS receptor complexes related to apoptosis, proliferation, and telomere activity.

Keywords: Apoptosis; Genistein; hTERT; Estrogen receptor; POT1

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Introduction

Steroid hormone nuclear receptors and their hormonal ligands are the key mediator groups in the endocrine signalling pathway, which play an important role in the regulation of differentiation, growth, and metabolic homeostasis. Estrogen regulates differentiation and maintains reproductive tissues, muscles, and other tissues by activating their receptors.¹ Ligands of estrogen receptors (ER) induce ER bonding at specific DNA response elements to regulate target gene expression. Receptors that can bind to DNA can positively or negatively regulate the transcription of target genes in specific cells or tissues. The ER agonist bonding in breast cancer cells can recruit a transcriptional co activator. In contrast, when it is antagonistic, the ER will actively recruit the corepressors, thereby causing transcriptional suppression of the target genes.² Structural studies indicate that estrogen and the antagonist estrogen compounds can induce different conformational changes in estrogen receptor-alpha (ER α), which may further determine the recruitment of co activators or corepressors, thus leading to diverse biological effects. Estrogen also acts as a mitogen to increase cell proliferation in both normal and cancerous breast cells, where ER α is a prognostic marker and a therapeutic target for breast cancer.³ One of the anti-estrogen compounds is methyl-piperidino-pyrazole (MPP) dihydrochloride (C₂₉H₃₁N₃O₃·2HCl), which is selective and has a high antagonist affinity to ER α compared with estrogen receptor-beta (ER β) (200 \times).

The most potential estrogen produced by human body is 17 β -estradiol. There are two estrogen metabolites, estrone and estriol. Although both are high affinity ligands, they have lower agonist properties against ER.⁴ Cellular signalling of estrogen is mediated through two subtypes of ER, namely ER α and ER β , both of which belong to the nuclear receptor transcription factor family. The most conserved domain of ER is the DNA-binding domain (DBD) involved in the introduction and binding of DNA, while the ligand binding occurs in the ligand-binding domain (LBD) at the COOH terminal region. Transcription activation is facilitated by two different activation (AF) functions,

i.e. AF-1 which is continuously active and located at the NH2 terminal of the receptor, and AF-2 located at the COOH terminal of LBD.⁵ ER α and ER β have a high degree of homology based on sequence alignment, except at the terminal NH2 domain. They also have similar affinity levels towards estrogen and bind to the same DNA response elements.⁶ Estrogen signalling begins when estrogen binds to ER where the transcriptional response depends upon the composition of the corrugating protein and the characteristics of the target gene promoter. The ER complex binds to the estrogen-responsive element (ERE) of the target gene, which subsequently recruits various cofactors, inducing the activation or repression of the target genes.⁷

Nitric oxide (NO) is produced by eNOS, which is a free radical involved in various cellular processes. Several studies have reported that activated eNOS can translocate to the nucleus upon binding to ER β .⁸ The eNOS/ER β complex determines chromatin remodelling, thus inducing the transcriptional activation of some prognostic genes, including hTERT, MSH2, CyclinD1, and ps2. These genes are quite sensitive to estrogen stimulus or intracellular NO levels.⁹

Epidemiological reports show that the consumption of food items that contain a lot of phytoestrogens is thought to be associated with a reduced risk of cancer due to hormonal induction.¹⁰ Studies have also reported the antiproliferative effect of genistein a phytoestrogen compound found most abundantly in soybean. Genistein has a higher affinity for ER β compared with ER α (9 \times).¹¹

Anti-estrogen designed to inhibit ER α signalling are widely and effectively being used clinically for the treatment of breast cancer.¹² However, these drugs have undesired side effects on non-target tissues, and long-term treatments render the cancer resistant to the anti-estrogen therapy. The idea of using ER β agonist offers new possibilities for pharmacological intervention in cancer therapy,¹³ and because of its specificity towards ER β , genistein is expected to have no undesirable side-effects upon substitution with estrogen. This study aimed to investigate the modulation of estrogen receptors by anti-estrogen and roles of genistein against the transcription process regulation of genes involved in the proliferation, apoptosis, and telomere activities.

Materials and Methods

Search for nucleotide and protein sequences

The structures of the components of genistein (CID: 5280961), 17 β -estradiol (CID: 5757), and MPP dihydrochloride (CID: 45073474) were obtained from PubChem Open Chemistry Database. The sequences of the proteins ER α (GI: 11907837), ER β (GI: 2970564), eNOS (GI: 266648), and nucleotides BCLX gene promoter sequence (GI: 488120), Casp3 (GI: 27979118), CyclinD1 (GI: 483600), Ki-67 (GI: 124550), hTERT (GI: 4239869), and POT-1 (GI: 649107630) were obtained from sequence database of the National Center for Biotechnology Information (NCBI), United States National Library of Medicine (NLM), and

National Institute of Health (NIH) (<http://www.ncbi.nlm.nih.gov>).

3D structure modelling of DNA, proteins, and bioactive components

The 3D structure modelling of ER α , ER β , and eNOS was performed to predict the 3D conformations using SWISS-MODELLER web server by homology modelling.^{14,15} The 3D model of the respective protein was then validated using Ramachandran Plot analysis. The 3D structures of BCLX, Casp3, CyclinD1, Ki-67, hTERT, and POT-1 gene promoters were predicted by 3D DART web server.¹⁶ The conversion of *.sdf files into *.pdb files from genistein, 17 β -estradiol and MPP dihydrochloride was performed using OpenBabel software.¹⁷

Docking computation

Simulation of docking between genistein compounds, 17 β -estradiol and MPP dihydrochloride on ER α and ER β , as well as the docking between the ER β + genistein/17 β -estradiol + eNOS complexes in the target gene promoters were performed using HEX 8.0 software.¹⁸ The docking protocol consists of three stages of visualization, namely rigid-body energy minimization, semi-flexible repair, and finishing refinement in explicit solvent. After the execution of each stage, the docking confirmation was scored and sorted based on the scoring function to facilitate the best conformation selection to be used at a later stage.

Inter-protein interaction analysis

The results of the next docking analysis will be visualized using Discovery Studio 4.1, LigPlot +, and Chimera 1.6.2.¹⁹ Analysis of the interaction between the protein complexes of ER β + genistein/17 β -estradiol + eNOS with the target gene promoter was done by using NUCPLOT software. Analyses of the protein–protein and protein–DNA interactions were performed to visualize the hydrogen bonding, hydrophobic bonding, and van der Waals bonding. Pharmacophore analysis was also performed to see the residues directly involved in the interaction process, minimization energy analysis was performed to improve the structure and shape of the molecules during interaction.

Results

Genistein exhibits a selective affinity for ER β

In this study, it was reported that genistein has a higher affinity for ER β compared to ER α . The docking analysis showed that for the genistein-ER α interaction takes lesser energy (–216.18 kJ/mol) as compared to the genistein-ER β interaction (–213.62 kJ/mol). However, the difference in energy is not significant enough. In addition, the docking analysis shows that the interaction between ER α with genistein does not involve formation of a chemical bond, because of which, the resultant unstable interaction may not be effective. Conversely, upon interacting with ER β , two hydrogen bonds and four hydrophobic bonds between

Table 1: Possible interactions between 17 β -estradiol and genistein with ER α or ER β .

Molecule	Point interaction	Category	Donor atom	Acceptor atom	Binding energy
ER α – 17 β -estradiol	ARG261:H – 17 β -estradiol:C	Hydrophobic bond	H	C	–218.31 kJ/mol
	PHE310:H – 17 β -estradiol:C	Hydrophobic bond	H	C	
	LEU311:H – 17 β -estradiol:C	Hydrophobic bond	H	C	
ER α – genistein	–	–	–	–	–216.18 kJ/mol
ER β – 17 β -estradiol	MET296:O – 17 β -estradiol:O	Hydrophobic bond	O	O	–207.90 kJ/mol
	THR299:H – 17 β -estradiol:C	Hydrophobic bond	H	C	
	LYS300:O – 17 β -estradiol:C	Hydrophobic bond	O	C	
	ASP303:H – 17 β -estradiol:C	Hydrophobic bond	H	C	
	VAL485:O – 17 β -estradiol:O	Hydrophobic bond	O	O	
ER β – genistein	LYS304:HZ3 – genistein:O	Hydrogen bond	HZ3	O	–213.62 kJ/mol
	VAL485:H – genistein:O	Hydrogen bond	H	O	
	MET296:O – genistein:O	Hydrophobic bond	O	O	
	THR299:O – genistein:O	Hydrophobic bond	O	O	
	VAL485:O – genistein:O	Hydrophobic bond	O	O	
	LEU490:O – genistein:O	Hydrophobic bond	O	O	

genistein and amino acid residues Lys304, Val485, Met296, Thr299, Val 485, and Leu490 of ER β are formed, leading to a stronger interaction (Table 1 and Figure 1).

Genistein has a higher affinity level in ER β compared to 17 β -estradiol

Table 1 and Figure 1 show the results of docking analysis. It can be clearly seen that the energy required by genistein for binding to ER β is lower (-213.62 kJ/mol) than that required by 17 β -estradiol (-207.90 kJ/mol). Docking analysis also

showed that the interaction between genistein and ER β was mediated by two hydrogen bonds and four hydrophobic bonds; whereas the interaction between 17 β -estradiol and ER β was mediated by five hydrophobic bonds only.

MPP dihydrochloride can inhibit the 17 β -estradiol binding with ER α

The analysis was conducted to see the counter mechanism of MPP dihydrochloride against ER α . It was seen that MPP

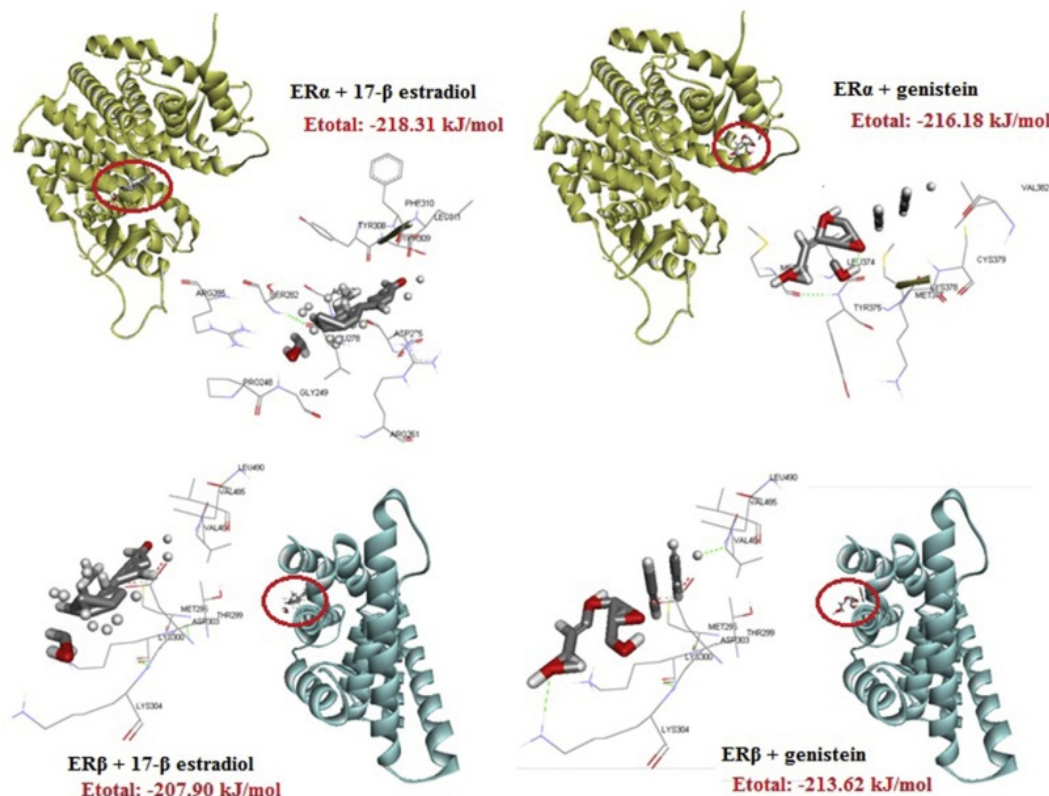


Figure 1: Analysis of docking and bond energy between estrogen receptor, 17 β -estradiol, and genistein. Genistein more easily interacts with both estrogen receptors than the 17 β -estradiol interactions with estrogen beta receptors.

Table 2: Possible interactions between MPP dihydrochloride and ER α .

Molecule	Point interaction	Category	Donor atom	Acceptor atom	Binding energy
ER α - MPP dihydrochloride	MPP dihydrochloride:N - ASP333:OD2	Hydrogen bond	N	OD2	-341.22 kJ/mol
	MPP dihydrochloride:H - LEU159:O	Hydrogen bond	H	O	
	MPP dihydrochloride:H - ARG326:O	Hydrophobic bond	H	O	
	ASP329:H - MPP dihydrochloride:C	Hydrophobic bond	H	C	
	GLN351:O - MPP dihydrochloride:C	Hydrophobic bond	O	C	
ER α , MPP dihydrochloride + 17 β -estradiol	-	-	-	-	-216.66 kJ/mol
ER α , MPP dihydrochloride + genistein	-	-	-	-	-205.45 kJ/mol

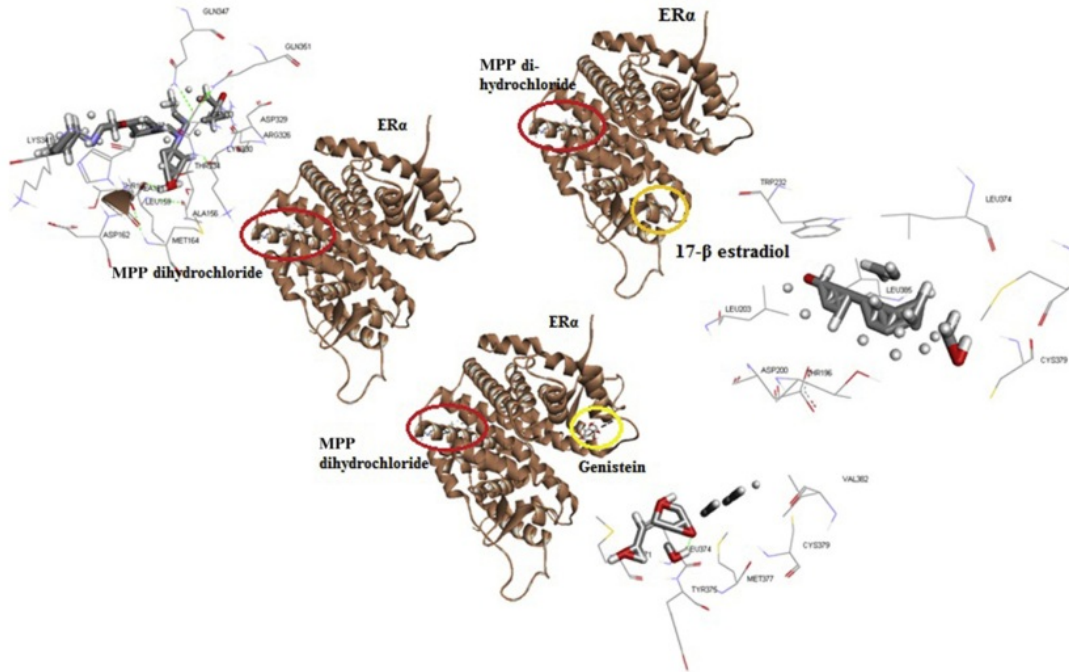


Figure 2: Analysis of docking and bond energy between estrogen alpha receptor, 17β-estradiol, genistein, and MPP-dihydrochloride.

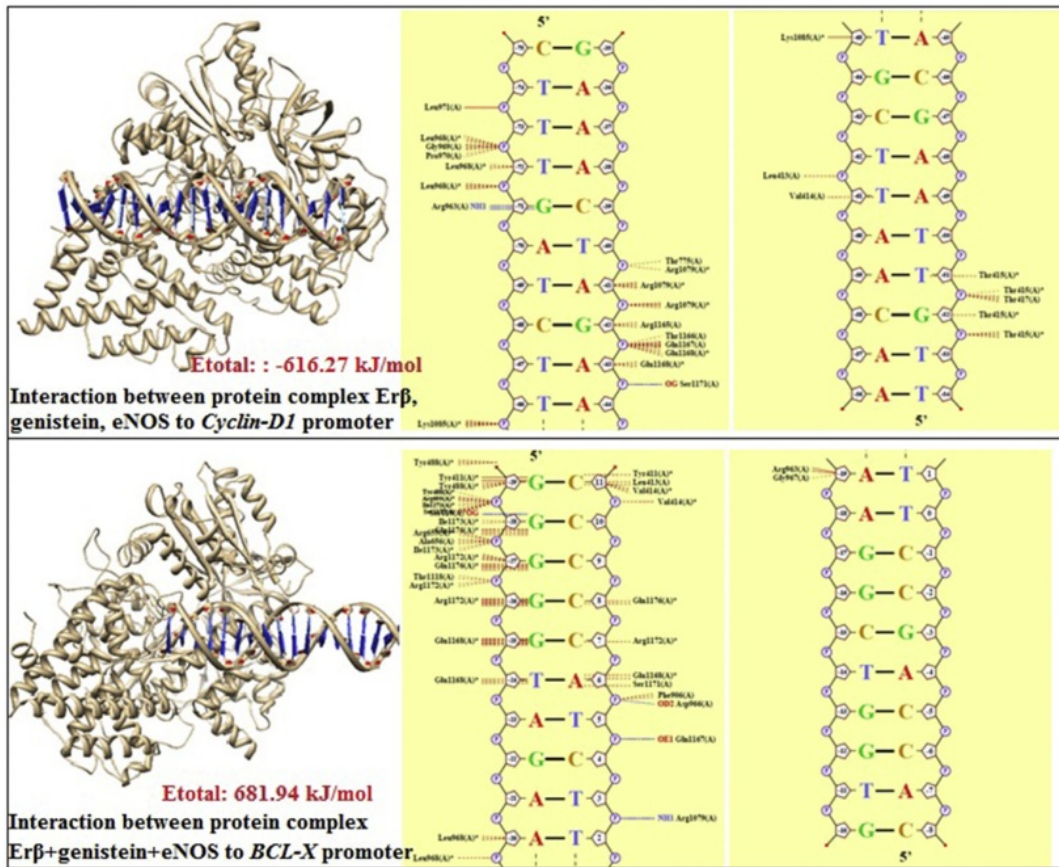


Figure 3: Analysis of docking and bond energy between estrogen beta receptor, genistein, eNOS, with Cyclin D1 or Bcl-X.

dihydrochloride interacted with ER α via two hydrogen bonds and three hydrophobic bonds via ER α residues Asp333, Leu159, Arg326, Asp329, and Gln351. Interaction sites of MPP dihydrochloride and 17 β -estradiol in ER α were differently located, but when MPP dihydrochloride binds to ER α , it brings about a change in 17 β -estradiol interaction site in ER α (Figure 2 and Table 2). Interaction analysis also shows that the interaction site changes may cause an increase in the energy required to bind, but the bonds which mediate the interaction are not formed at all. It is suspected that when MPP dihydrochloride binds to ER α , the ER α conformation changes so as to inhibit the 17 β -estradiol and ER α binding. Unlike with genistein, MPP dihydrochloride did not cause changes in the interaction site of genistein on ER α , but may cause the energy required for interaction become larger (-205.45 kJ/mol).

Genistein is more effective in inducing transcription of BCLX, Casp3, Ki-67, CyclinD1, hTERT, and POT1 genes

Out of the 6 genes analysed in this study, BCLX and Casp3 genes are involved in cell apoptosis, the Ki-67 and CyclinD1 genes are involved in cell proliferation, and hTERT and POT1 genes participate in the telomerase activity. The analysis showed that upon binding to genistein, the ER β /eNOS complex/would be/was induced easily by the

transcriptional activation of the above-mentioned genes. This is seen from the lower binding energy and higher number of the bonds formed (Figures 3 and 4, Table 3). In ER β , the DNA binding domain extends from residues 149–214, the steroid binding domain is between residues 215–530, while the Zinc finger is positioned at residues 149–169 and 185–209.

Discussion

Genistein is a phytoestrogen compound found mostly in soybean plant. In this study it was proven that the energy of the interaction between genistein and ER β was slightly lower than that with ER α . This indicates that genistein can interact with both receptors, but it is relatively easier in case of ER β . In addition, this type of bond with ER β shows a more stable bonding compared to ER α . This finding is consistent with the previous reports stating that genistein can bind to both of the estrogen receptor isoforms.^{20,21} In this study the interaction between genistein and Lys304, Val485, Met296, Thr299, Val 485, and Leu49 of ER β has been elucidated. This investigation also proved the genistein-17 β -estradiol interaction against ER β . The energy of the interaction and the type of bonds formed indicate that genistein has a higher affinity for ER β than 17 β -estradiol. The hydrogen bonding is stronger as compared to the hydrophobic

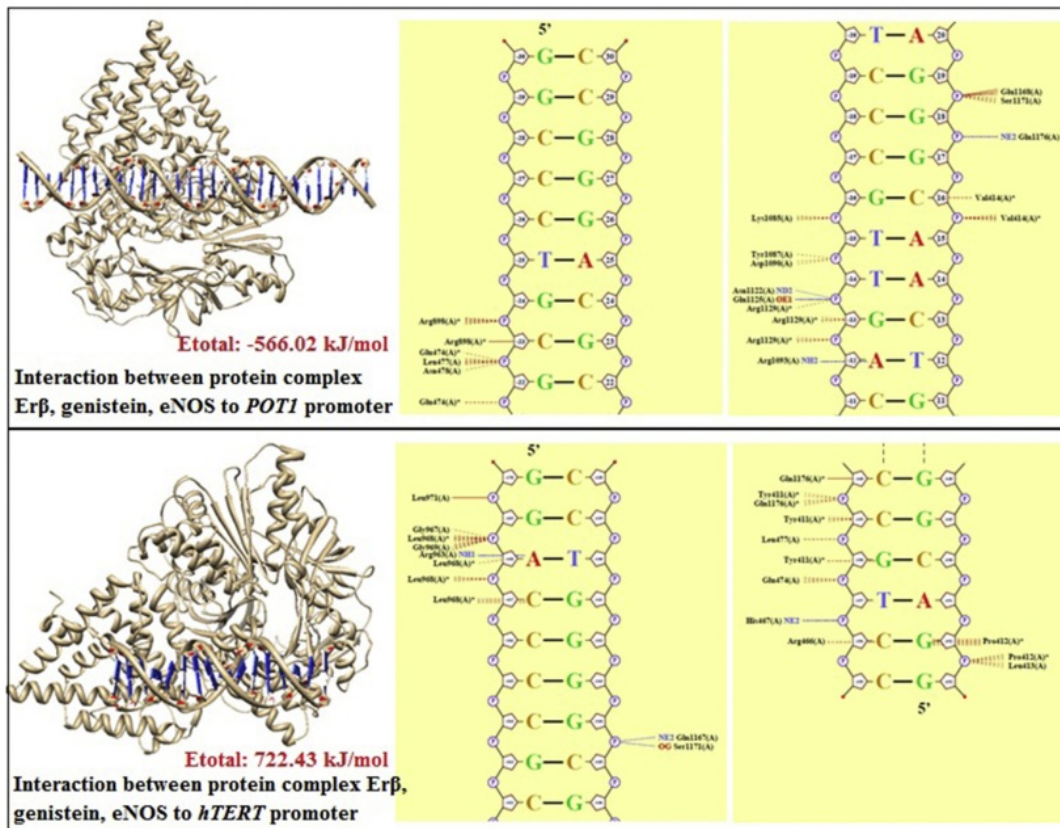


Figure 4: Analysis of docking and bond energy between estrogen beta receptor, genistein, eNOS, with POT1 or hTERT.

Table 3: Interactions of ER β /eNOS/genistein-17 β estradiol complexes on target gene promoters.

Molecule	Number of interaction	Point interaction	Binding energy
ER β , 17- β estradiol, eNOS + <i>BCLX</i>	27 (4 hydrogen bonds, 23 van der waals interaction)	G(-29A) \rightarrow Gln1167, Ser1171, Glu1168; G(-25A) \rightarrow Ser 619; A(-23A) \rightarrow Pro621, Ser617, Ser624; G(-22A) \rightarrow Ser617, Ile616, Ser625; C(10B) \rightarrow Glu1168; C(9B) \rightarrow Arg1172, Asp620, Thr1118, Thr1121, Asn1122; C(8B) \rightarrow Ser619, Gln1176, Ser619, Arg655, Ala656; C(7B) \rightarrow Cys618, Ser619, Arg655, Ile1173, Ser1177; C(6B) \rightarrow Gln1176.	-543.07 kJ/mol
ER β , genistein, eNOS + <i>BCLX</i>	36 (4 hydrogen bonds, 32 van der waals interaction)	G(-29A) \rightarrow Tyr488, Tyr411, Arg655, Ile1173, Ser1177; G(-28A) \rightarrow Ser619, Ile1173, Gln1176, Arg655, Ala656, Ile1173; G(-27A) \rightarrow Arg1172, Gln1176, Thr1118, Arg1172; G(-26A) \rightarrow Arg1172; G(-25A) \rightarrow Glu1168; T(-24A) \rightarrow Glu1168; A(-20A) \rightarrow Leu968; A(-19A) \rightarrow Arg963, Gly967; C(11B) \rightarrow Tyr411, Leu413, Val414; C(8B) \rightarrow Gln1176; C(7B) \rightarrow Arg1172; A(6B) \rightarrow Glu1168, Ser1171, Phe906, Asp966; T(5B) \rightarrow Gln1167; T(3B) \rightarrow Arg1079	-681.94 kJ/mol
ER β , 17- β estradiol, eNOS + <i>Casp3</i>	23 (4 hydrogen bond, 19 van der waals interaction)	T(-121A) \rightarrow Ser1171; G(-115A) \rightarrow Ser617, ys618, Pro621, Ser624; C(-114A) \rightarrow Ser617; A(-81B) \rightarrow Glu1168; A(-83B) \rightarrow Arg1172, Asp620, Thr1118, Thr1121; G(-84B) \rightarrow Ser619, Asp620, Arg1172, Arg655, Arg656; C(-85B) \rightarrow Ile1173, Gln1176, Arg655, Gln1176, Ser1177.	-522.93 kJ/mol
ER β , genistein, eNOS + <i>Casp3</i>	16 (one hydrogen bond, 15 van der waals interaction)	G(-115A) \rightarrow Ala1098; C(-114A) \rightarrow Thr1094, Glu1095; G(-107A) \rightarrow Val414; A(-106A) \rightarrow Ser619; G(-105A) \rightarrow Glu1168, Arg1172; C(-104A) \rightarrow Glu1168; (C(-91B) \rightarrow Arg1093; G(-92B) \rightarrow Arg1093, Gln1125, Thr1126, Arg1129; C(-93B) \rightarrow Gln1089, Asp1090; C(-94B) \rightarrow Lys1085.	-636.42 kJ/mol
ER β , 17- β estradiol, eNOS + <i>Ki-67</i>	21 (4 hydrogen bond, 17 van der waals interaction)	G(-41A) \rightarrow Gln1176, Ser1177; G(-40A) \rightarrow Ser619, Arg655, Ile1173; G(-39A) \rightarrow Ser619, Asp620, Thr1121; C(-38A) \rightarrow Asp620, Arg1172, Asn1122; T(-37A) \rightarrow Arg1172; C(-25B) \rightarrow Ile616, Ser617, Ser624, Ser625; G(-26B) \rightarrow Cys618, Ser624; C(-32B) \rightarrow Glu1168.	-546.92 kJ/mol
ER β , genistein, eNOS + <i>Ki-67</i>	29 (5 hydrogen bond, 24 van der waals interaction)	C(-44A) \rightarrow Arg1049, Arg1079; C(-42A) \rightarrow Gln1167; G(-41A) \rightarrow Phe906, Asp966, Ser1171; G(-40A) \rightarrow Glu1168, Ser1171; G(-39A) \rightarrow Arg1172; G(-22B) \rightarrow Arg963; G(-23B) \rightarrow Arg963, Gly967, Leu968; C(-28B) \rightarrow Glu1168; C(-29B) \rightarrow Glu1168; G(-30B) \rightarrow Arg1172, Thr1118; A(-31B) \rightarrow Arg1172, Gln1176, Arg655, Ala656, Ile1173; C(-32B) \rightarrow Ser619, Ile1173, Arg655, Ile1173, Ser1177.	-638.37 kJ/mol
ER β , 17- β estradiol, eNOS + <i>CyclinD1</i>	26 (3 hydrogen bond, 23 van der waals interaction)	C(-75A) \rightarrow Ser1171; T(-74A) \rightarrow Arg1172; T(-69A) \rightarrow Ser617, Cys618, Pro621, Ser617, Ser624; C(-68A) \rightarrow Ser617, Ile616; A(-36B) \rightarrow Glu1168; A(-37B) \rightarrow Arg1172, Asp620, Thr1118, Thr1121; A(-38B) \rightarrow Ser619, Asp620, Arg1172, Gln1176, Arg655, Ala656; C(-39B) \rightarrow Ile1173, Gln1176, Arg655, Ile1173, Gln1176, Ser1177.	-542.26 kJ/mol
ER β , genistein, eNOS + <i>CyclinD1</i>	26 (2 hydrogen bond, 24 van der waals interaction)	T(-74A) \rightarrow Leu971; T(-73A) \rightarrow Leu968, Gly969, Pro970; T(-72A) \rightarrow Leu968; G(-71A) \rightarrow Arg963; T(-66A) \rightarrow Lys1085; T(-65A) \rightarrow Lys1085; T(-62A) \rightarrow Leu413; T(-61A) \rightarrow Val414; T(-40B) \rightarrow Thr775, Arg1079; A(-41B) \rightarrow Arg1079; G(-42B) \rightarrow Arg1165, Thr1166, Gln1167, Glu1168; A(-43B) \rightarrow Glu1168, Ser1171; T(-51B) \rightarrow Thr415, Thr417; G(-52B) \rightarrow Thr415.	-616.27 kJ/mol
ER β , 17- β estradiol, eNOS + <i>hTERT</i>	20 (5 hydrogen bond, 15 van der waals interaction)	G(-163A) \rightarrow Arg764, Ser765; C(-162A) \rightarrow Ser765, Arg782; C(-161A) \rightarrow His949; C(-160A) \rightarrow Glu1033; C(-159A) \rightarrow Thr948, Glu1033; G(-158A) \rightarrow Ser947; C(-150B) \rightarrow Ser947, Pro950, Gly787, Gly951; A(-151B) \rightarrow Pro946, Pro950, Gln792, Tyr793, Pro946; G(-152B) \rightarrow Ser448, Gln794.	-611.79 kJ/mol
ER β , genistein eNOS + <i>hTERT</i>	22 (4 hydrogen bond, 18 van der waals interaction)	G(-170A) \rightarrow Leu971; G(-169A) \rightarrow Gly967, Leu968, Gly969.; A(-168A) \rightarrow Arg963, Leu968; C(-167A) \rightarrow Leu968; C(-160A) \rightarrow Gln1176, Tyr411, Gln1176; C(-159A) \rightarrow Tyr411, Leu477; G(-158A) \rightarrow Tyr411, Glu474; T(-157A) \rightarrow His467; C(-156A) \rightarrow Arg466; G(-144B) \rightarrow Gln1167, Ser1171; G(-152B) \rightarrow Pro412, Leu413.	-722.43 kJ/mol
ER β , 17- β estradiol, eNOS + <i>POT1</i>	43 (5 hydrogen bond, 38 van der waals interaction)	C(-21A) \rightarrow Trp345, Asp349; T(-20A) \rightarrow Trp345, Asp349, Ile348, Phe387; C(-19A) \rightarrow Asp349, Arg388, Glu389; C(-18A) \rightarrow Asp349, Ala589, Glu592, Met593; C(-17A) \rightarrow Ser638; G(-16A) \rightarrow Asp637, Ser638; T(-15A) \rightarrow Glu562, Asp637; G(-10A) \rightarrow Ala515; T(-9A) \rightarrow Ala515; A(20B) \rightarrow Asn598; C(16B) \rightarrow Lys353; A(15B) \rightarrow Lys353, Glu562, Gly352, Lys353, Asp363; C(13B) \rightarrow Glu562, His563, Glu564; T(12B) \rightarrow Thr644.	-494.65 kJ/mol

(continued on next page)

Table 3 (continued)

Molecule	Number of interaction	Point interaction	Binding energy
ER β , genistein eNOS + <i>POT1</i>	20 (4 hydrogen bond, 16 van der waals interaction)	G(-24A) \rightarrow Arg898; C(-23A) \rightarrow Arg898, Glu474, Leu477, Asn478; G(-22A) \rightarrow Glu474; G(-16A) \rightarrow Lys1085; T(-15A) \rightarrow Tyr1087, Asp1090; T(-14A) \rightarrow Asn1122, Gln1125, Arg1129; G(-13A) \rightarrow Arg1129; A(-12A) \rightarrow Arg1093; G(19B) \rightarrow Glu1168, Ser1171; G(18B) \rightarrow Gln1176; C(16B) \rightarrow Val414; C(10B) \rightarrow Thr1094, Glu1095; A(9B) \rightarrow Ala1098.	-566.02 kJ/mol

bonding. The stronger the bonds formed between the two molecules, the higher the stability of the interactions. Hence, it is suspected that genistein has a stronger affinity towards ER β if the affinity levels between 17 β -estradiol and ER β are to be compared. The study supports the previous findings that genistein-ER β interactions involve the -OH groups of His475, Glu305, and Arg306.²²

Methyl-piperidino-pyrazole (MPP) is a very selective ER α antagonist. Its affinity for ER α is 200 times as compared to ER β .²³ Our study revealed that MPP dihydrochloride could inhibit the interaction between 17 β -estradiol and ER α . The inhibitory mechanism is supposedly through the conformational changes in ER α , thus rendering 17 β -estradiol non-functional for effective binding to ER α . MPP dihydrochloride also blocks the interaction between genistein and ER α by increasing the energy required for the interaction to occur.

Both ER α and ER β can be activated by estrogen, wherein both their ligand-binding domains allow for the selection of binding ligands. DNA-binding domains of ER α and ER β have high conservation rates and both share between 46% and 73% of chromatin binding sites.²⁴ Although several clinical studies linked the ER β expression to better clinical outcomes,^{25,26} several other reports show an association between ER β expression and the Ki-67 proliferation marker.^{27,28} From our results of, it is speculated that ER β can indeed induce the expression of Ki-67 as seen from the interaction between ER β /eNOS/17 β -estradiol-genistein with the Ki-67 gene promoter. In addition, it is suspected that genistein is more effective in inducing the occurrence of such interactions as compared to 17 β -estradiol. This was seen from the lower energy required for interaction and the greater interaction of hydrogen and van der-Waals formed. Another proliferation marker analysed in this study is the CyclinD1 gene. This study reported that the ER β /eNOS/17 β -estradiol-genistein complex may also induce transcriptional activation of the CyclinD1 gene. The results from this study are not consistent with a previous study which reports that ER α is an activator of the CyclinD1 gene, whereas ER β is its repressor.²⁹

Different co activator/co repressor proteins determine the transcription complex to be recruited, thus causing differential gene transcriptions depending on the type of co regulator ER-complex dimer.³⁰ We also evaluated the effect of formation of estrogen ligand complexes, and impact of eNOS on the promoter region of apoptotic and telomere activity genes. We revealed here that genistein ligand increases the ease and stability of interactions than the ligand estradiol indicating that genistein administration can

increase the genomic activity of estrogen-eNOS receptor complexes related to apoptosis and telomere activity.

It was concluded that administration of genistein can increase the genomic activity of oestrogen-eNOS receptor complexes related to apoptosis, proliferation, and telomere activity.

1 Conflict of interest

The authors have no conflict of interest to declare.

Ethical approval

3 This research has received ethical approval from the Research Ethics Committee, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia.

Authors' contributions

HY, EH, TN, and HK conceived and designed the study, conducted research, provided research materials, and collected and organized data. HY analysed and interpreted data. HY, EH, and TN wrote the initial and final drafts of the article. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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