Trop J Nat Prod Res, March 2024; 8(3):6624-6630 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

The Potential Health Benefit of Cowpea Estrogen-like Activity to Restore Vaginal Epithelial Cells Thinning Due to Menopausal Syndrome

An Nisa Fithri[1]*, Yuyun Yueniwati[3], I Wayan Arsana[4], Husnul Khotimah[5], Wiwit Nurwidyaningtyas[6,7]

[1]*Doctoral Program of Medical Sciences, Faculty of Medicine, Universitas Brawijaya, Indonesia*
[2]*Midwifery Program, STIKES Kendedes, Malang, Indonesia*
[3]*Department of Radiology, Saiful Anwar Hospital, Faculty of Medicine, Universitas Brawijaya, Indonesia*
[4]*Department of Fertility, Endocrinology and Reproduction, Obstetrics and Gynecology, Saiful Anwar Hospital, Faculty of Medicine, Universitas Brawijaya, Indonesia*
[5]*Department of Pharmacology, Faculty of Medicine, Universitas Brawijaya, Indonesia*
[6]*Center for Biomedical Research, Research Organization for Health, BRIN, Cibinong Science Center, Bogor, West Java, Indonesia*
[7]*Department of Molecular and Cellular Biology, STIKES Kendedes, Malang, Indonesia a*

ARTICLE INFO

Article history:
Received 14 February 2024
Revised 11 March 2024
Accepted 18 March 2024
Published online 01 April 2024

ABSTRACT

Vigna unguiculata, cowpea, contains genistein, a compound with a structure similar to estrogen. However, the genistein content of cowpea and its potential for addressing menopause-related vaginal epithelial cell atrophy remains uncharted. This research aims to examine the interaction mode and estrogenic effect of Indonesian cowpea seeds' genistein on the estrogen receptor (ER) both *in silico* and *in vivo*. Through the application of liquid chromatography-tandem mass spectrometry (LC-MS/MS), the genistein content of dry pasta cowpea seeds KT4 and KT6 was examined. Autodock 4.0, an integral component of the PyRx 0.9.5 software, was utilized to establish a virtual interaction between genistein molecules from cowpea compounds and the ERα and ERβ proteins. An immunohistochemistry assay was employed to characterize the estrogen receptor expression profile of vaginal tissue in 36 female Sprague-Dawley rats subjected to treatment-dependent doses of cowpea. Genistein was 15.64 µg/g higher in cowpea KT4 than in KT6. Moreover, genistein docking in cowpeas revealed a significant interaction with ERα and ERβ at -9.03 and 10.8 kcal/mol, respectively. Expression of ERα and ERβ significantly increased in vaginal menopausal rat models treated with 4-ethanolic extract of cowpea seed varieties at a dose of 5 mg/kgBB compared to a lower dose (p=0.004). In sum, cowpea ethanol extract exhibited to have an estrogenic effect on vaginal tissue in menopausal models. Accordingly, future applications of the cowpea as a complementary therapy for vaginal dryness due to menopausal syndrome will enhance the quality of life for postmenopausal women.

***Keywords*:** estrogen receptor, genistein, vaginal atrophy, *Vigna unguiculata*.

Introduction

Menopause, commonly characterized by menstrual cycle irregularities, night sweats, and hot flushes, refers to a physiological state that is not pathological in nature and signifies the permanent cessation of ovarian function.[1] Untreated symptoms in midlife can result in changes in quality of life, decreased work productivity, and potential health issues. Approximately 40% of women experiencing menopause report symptoms such as vaginal dryness and painful intercourse, which are attributed to decreased estrogen levels leading to vaginal atrophy.[2] In instances of vaginal atrophy, the reduction of endogenous estrogen leads to the inhibition of cell growth and concurrently induces a minor thinning of the vaginal wall epithelium. Previous research has indicated that the signaling pathways associated with E2 play a crucial role in modulating the formation of a physical barrier.

*Corresponding author. E mail: annisafithri124@gmail.com
Tel: +62 821-3910-4309

Citation: Fithri AN, Yueniwati Y, Arsana IW, Khotimah H, Nurwidyaningtyas W. The Potential Health Benefit of Cowpea Estrogen-like Activity to Restore Vaginal Epithelial Cells Thinning Due to Menopausal Syndrome. Trop J Nat Prod Res. 2024; 8(3):6624-6630. https://doi.org/10.26538/tjnpr/v8i3.22

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

This is achieved through the regulation of vaginal epithelial thickness and the facilitation of antimicrobial peptide synthesis through receptor-binding interactions.[3,4] Estrogen is commonly employed as a form of hormonal replacement therapy in the treatment of many illnesses, such as vaginal atrophy-related conditions in women experiencing menopause. The Women's Health Initiative (WHI) has highlighted concern regarding the lack of regular administration of estrogen replacement medication as a prophylactic measure for many patients.[5,6] Cowpeas contain genistein, a prominent isoflavone found in legume plants. Genistein is a natural flavonoid that has a strong affinity for the estrogen receptor (ER) due to its chemical structure resembling 17β-estradiol.[7,8,9] According to a previous study, genistein acts similarly to estrogen in living organisms and in lab settings, promoting mammary gland growth and boosting the growth of MCF-7 cell tumors in mice that have had their ovaries removed.[10,11] In support of this finding, our prior research has already established the correlation between estrogen treatment and the promotion of vaginal epithelial cell repair following ovariectomy (OVX) through key regulatory genes.[12] Moreover, a positive correlation was demonstrated between the initiation of menopausal hormone therapy and the occurrence of Alzheimer's disease and all-cause dementia, even among women who initiated treatment at the age of 55 years or younger. Additionally, the potential negative consequences associated with extended estrogen therapy encompass vaginal hemorrhage, perineal pain, and the development of breast cancer. [13, 14, 15] Consequently, further research is required to identify alternative agents capable of producing similar advantageous outcomes to estrogen with minimal or no adverse effects.

However, the diversity of phytochemicals in cowpeas is also controlled by environmental interactions that are often overlooked.[16,17,18] To date, no research has investigated variations in genistein levels among various cowpea varieties. Thus, the identification of genistein in cowpea variants presents a distinctive opportunity for our future investigation and its biological significance in the OVX models of vaginal epithelial cells, leading to the significance of this research which is to observe and develop the comparable models wherein the ovarium was excluded, in order to examine the impact of cowpea on the preservation of the estrogen receptor in menopausal models.

Materials and Methods

Ethics approval

The protocols and procedures for handling animals were conducted following the guidelines established by the Animal Care and Use Committee of Universitas Brawijaya and complied with the authorized protocol of the Ethics Committee of Brawijaya University of Medical Faculty No. 106-KEP-UB-2020. The experiments were conducted using mice that were kept in a controlled environment with regulated temperature and humidity and were provided with unrestricted access to water and food.

Sample collection and preparation

The genotype CES 41-6, referred to as KT4, and the line code 191/VITA4/91-B-33, also identified as KT6, of cowpea seeds (*Vigna unguiculata*) were acquired from the Research Institute of Various Beans and Tubers (Balitkabi) in Malang. The extract was subjected to evaporation using an oven set at a temperature range of 37-40°C. The extract that was acquired was processed for subsequent analysis using LCMS. The extract results were evaporated using a Binder drying oven E28 series (No.cat Z630772, Merck SK Germany) at 37-40°C. The obtained extract was prepared for further tests using Qsight 220 triple Quad LCMS/MS (PerkinElmer Inc).

Analysis of Genistein Component by LC-MS/MS.

The extracted samples were subjected to analysis using different amounts of methanol solvent, specifically 10 ng/mL, 20 ng/mL, 30 ng/mL, 40 ng/mL, and 80 ng/mL. The formulation of the mobile phase was the combination of 0.1% formic acid (A) with acetonitrile and 0.1% formic acid in methanol (B). The operational parameters of the LC, consisting of a vacuum gas eliminator, quadrupole pump, and thermostatic automated sampler, were regulated using the x-Calibur 2.1 application. The flow rate of the mobile phase was set at 300 µL/min, while the mounting parameters were as follows: 0.0-1.0 min 80%b, 1.0-3.5 min 20%b, 3.5-5.0 min 20%b, and 5.5-7.0 min 80%b. The shaft used was Hypersil Gold specification (50 mm x 2.1 mm x 1.9 µm). The injection volume in the LC is 1 µL. The column temperature was maintained at 30°C, while the sample chamber temperature was adjusted to 16°C. The analysis of the data was conducted utilizing the MzCloud MS/MS library. The qualitative and quantitative determination of Genistein Cowpea seeds (*Vigna unguiculata*) KT4 and KT6 was conducted using the LC-MS/MS method, using previously established protocols. The quantification process involved determining the peak area of the genistein complex by comparison with the standard.

Protein prediction and network analysis

The researchers conducted a structure similarity analysis to assess the degree of similarity between the test compounds and the medication database. A comparative analysis of cowpea chemicals was conducted using SIMCOMP (similar complex) software, available at https://www.genome.jp/tools/simcomp/. The group similarity approach (SEA) was utilized to determine the target protein. The MaxTC value, also known as the Tanimoto coefficient or high coefficient, quantifies the similarity between input compounds (horsetail beans) and database compounds. The cutoff for the MaxTC score (Tanimoto coefficient) is set at 0.7.

Compilations of the target proteins from both databases were incorporated into the STRING database's (https://string-db.org) list of multiple proteins. Results from the visualization are saved in a TSV file format and then imported into Cytoscape v.3.9.0.

Molecular docking analysis to predict protein targets

A specific docking, Autodock 4.0, which was integrated into PyRx 0.9.5, was utilized in the docking procedure to simulate the interaction between the control agonist and the receptor. Protein preparations and liganding were executed utilizing PyMol 2.5.1. Energy reduction was conducted by configuring Plugin Open Babel PyRx with the UFF field strength energy reduction parameters. Utilizing Lamarckian Genetic Algorithm Parameters, docking was performed. The Genetic Algorithm is capable of executing 50,150 models with a maximal power of 2,500,000. The receptor proteins utilized in this study were as follows: 1) the estrogen receptor alpha complexed with the genistein crystal structure (PDB ID 1X7R); and 2) the ER-beta bound estradiol crystal structure (PDB ID 5TOA) in relation to the cowpea ligand with the highest similarity value, Daidzein (ID Pubchem 5281708), Genistein (ID Pubchem 5280961), and Glycitein (Pubchem ID 5317750).

Predictions based on Lipinski's rule of five aid in differentiating drug-like molecules from non-drug-like molecules or in determining drug propensity. The SWISSADME database (http://www.swissadme.ch/) was utilized to obtain these predictions from a compound. The similarity of a compound to a drug allowed Lipinski to forecast whether its metabolism would be successful or unsuccessful.

Animal groups

The pharmacology laboratories of the Medical Faculty, Universitas Brawijaya, Indonesia, provided permission for the unrestricted provision of food and water and the housing of 18 female Sprague-Dawley rats, aged 18 months (n = 18; weighing 300 g). The rats were maintained under standard lighting conditions. The implementation of standardized protocols was employed to perform bilateral ovariectomy or placebo surgery. In accordance with the authorized guidelines, ketamine hydrochloride and xylocaine were utilized to induce anesthesia in the animals.[19,20] A dorsal midline skin incision was performed on both sides, except for the placebo group, to remove the ovaries after shaving off the fur. After twenty days of observation and before estradiol treatment, the ovariectomized (OVX) animals were individually confined in separate containers.

A cohort of OVX rats received estrogen for two weeks via subcutaneous implantation of silastic tubes (Dow Corning, Midland, Michigan) containing 0.1% 17β-estradiol in ethanol. All other groups received the vehicle treatment only (deionized water) Rats were divided into the following groups (n=6) for this study: (i) placebo-operated, (ii) OVX, and (iv) OVX-estradiol treatment. The exclusion of subjects occurred in the event of mortality or disease.

Tissue Processing, Embedding and Sectioning

The posterior part of the vagina was sampled for vaginal tissue, which was subsequently sectioned to a diameter of 1 cm and a thickness of approximately 3 mm. Before being incubated at room temperature for approximately forty-eight hours, the vaginal specimens were fixated with a 10% neutral buffer formalin solution. 10% Buffered Formalin (catalog number HT501128-4L, Sigma-Aldrich, Merck & Co., Inc., USA) was used to stabilize and soak the sample. Following tissue processing overnight with the Leica TP1020 (USA), the samples were subsequently imbedded in molten paraffin wax applying the HistoCore Arcadia H-Heated Paraffin Embedding Station (Leica, USA). Sections were cut at 4 µm rotary microtome (RM2235, Leica, USA). Paraffin filaments were dried at 60°C for 16 hours after being flattened in a water bath at 40°C after their placement on polysine microscopy slides (Nunc ™ Thermo Scientific, Sakura Heater, Tokyo, Japan).

ERα and ERβ Measurement

The experiment involved utilizing an Elabscience cat number E-IR-R221 3.3'-diaminobenzidine stain reagent (DAB) Super PlusTM High Sensitive and Rapid Immunohistochemical reagent for immunohistochemistry. Monoclonal antibody for Estrogen Receptor beta Antibody (B-1) and Estrogen Receptor alpha Antibody (F-10) with Santacruz Biotechnology cat number sc-8002 and sc-390243, respectively. The specimens that had been stained with HE and IHC were examined using an Olympus BX-53 light microscope (Olympus Corporation, Tokyo, Japan). The observation was conducted by

calculating the number of cells detected in 20 fields of view at a 1000x magnification; the dataset comprised approximately 1500 cells.

Statistical Analysis

The data was expressed as the mean squared with the standard deviation. A one-way analysis of variance (ANOVA) was conducted to perform statistical analysis, followed by a post hoc test to determine significant differences. A value of $p < 0.05$ was deemed to be statistically significant. The statistical analysis was conducted using SPSS 21.0 statistical software, developed by Chicago State University in Illinois, USA.

Result and Discussion

The present study unveiled novel findings regarding the function of cowpea ethanolic extract in preserving tissue integrity within the lower female reproductive system. The outcomes of this investigation have been synthesized using both in silico and in vivo menopausal models.

Cowpea Isoflavone

The LCMS analysis conducted on cowpea varieties KT4 and KT6 revealed the presence of several isoflavones, including Daidzein, Genistein, Glycetein, Daidzin, and Genistin, in both varieties. The results of this research indicate that genistein was discovered as the primary isoflavone ingredient in the two cowpea types that were implemented (Figure 1). Subsequently, to determine the cowpea variety with the highest genistein content, a comparative analysis was conducted on the genistein levels of the two varieties, as depicted in the results presented in Table 1.

In comparison to KT6, cowpea KT4 is richer in genistein. It is common knowledge that the isoflavone aglycone group, of which genistein (4', 5,7-trihydroxy isoflavone) was identified in both cowpea varieties, comprises processed foods including tempeh and soybean plants.[21] Genistein possesses a comparable structure to dienestrol and is an exact analog of diethylstilbestrol suggesting that estradiol exhibits a significant affinity at the ERα and ERβ, respectively.[22,23] The potential estrogen receptor agonism exhibited by genistein is a significant factor motivating our decision to conduct *in vivo* investigations. Furthermore, the epithelial ER is essential for the maintenance of the vaginal structure; its absence compromises the vaginal epithelium's structural integrity, which can irritate the sexual sphere. Furthermore, the administration of the ethanolic extract of cowpea resulted in the upregulation of estrogen receptors in the vaginal epithelium, which was an identical effect to the estrogen treatment that served as the control. Cowpea administration remains a promising candidate for the future development of a non-hormonal therapy for menopausal symptoms in women, additionally aiming to reduce the long-term effects linked to hormonal therapy for this group.

Prediction of bioactive compound interaction

The structure and atomic number of genistein are illustrated in Figure 2. A similarity score of 0.5 indicates that the molecular structure of Genistein resembles that of Dienestrol, a synthetic nonsteroidal estrogen, and Diethylstilbestrol, another synthetic nonsteroidal estrogen. In addition, a protein interaction docking test was conducted to ascertain the interaction model between different isoflavones derived from cowpea and estrogen receptors that are implicated in the repair pathway for vaginal epithelial cell atrophy. In determining the interpretation of docking outcomes, binding affinity is a representation of protein interactions. The degree of affinity between the ligand and the receptor increases as the binding affinity value decreases, while conversely, a greater binding affinity value results in a diminished affinity between receptors.

The results presented in Table 2 support the notion that genistein exhibits a generally higher affinity for the estrogen receptor alpha (ERα) subtype than for the regulator beta (ERβ) subtype. Daidzein exhibited the smallest binding affinity to serum estrogen receptor alpha. Conversely, all three varieties of isoflavones tested demonstrated an equivalent affinity to serum of ERβ; genistein, however, exhibited the lowest CID.

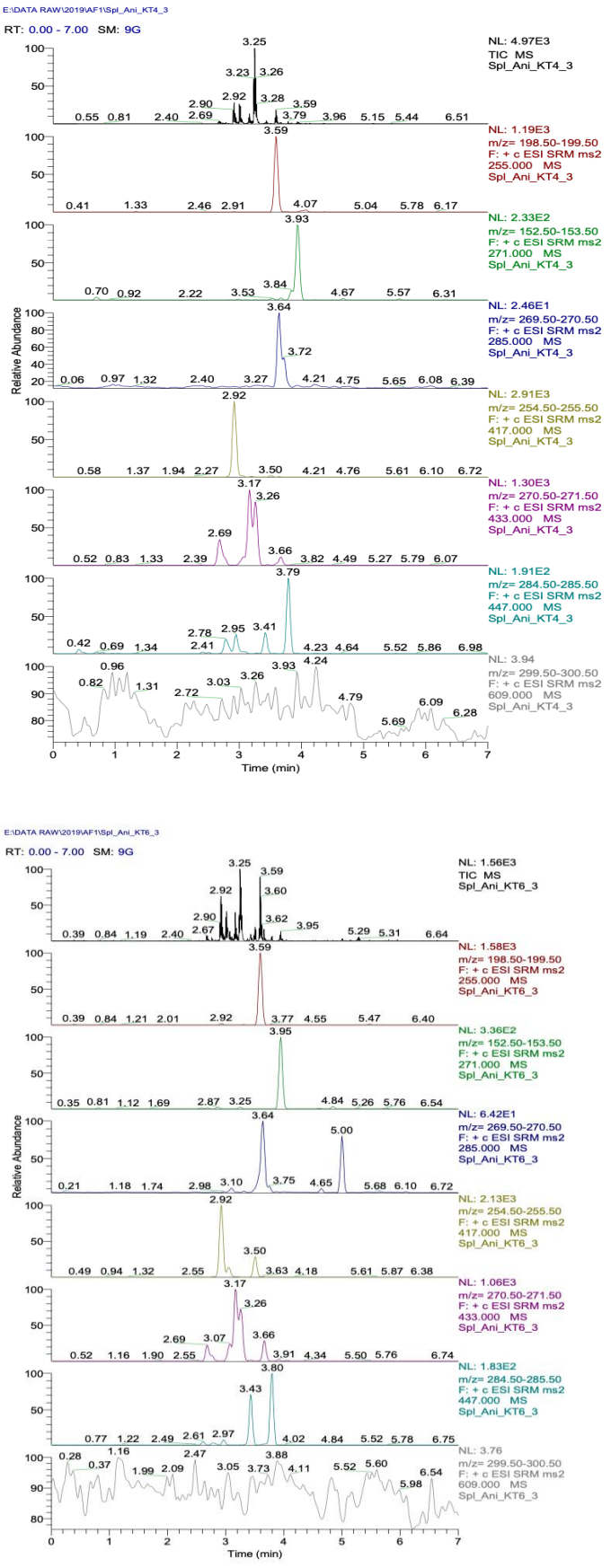

Figure 1: Isoflavone chromatogram of *Vigna unguiculata* (A) variety KT4; (B) KT6 variety

Table 1: Comparison of genistein content in cultivars KT 4 and KT 6

Variety	Sample weight (g)	Areas	Concentration (ng/ml)	Weight (ng)	Genistein levels (µg/g)
Cowpea KT4	0.0325	1.213	50.822	508.22	15.64
Cowpea KT6 (191/VITA4/91-B-33)	0.0542	1.372	55.304	553.04	10.55

g=gram, ng=nanogram, ml=milliliter, µg=microgram

Table 3 and Figure 3 illustrate the complex reaction between bioactive substances derived from cowpea and estradiol, which serves as the control. Amino acids such as Arg394/34, Leu384 (ERα)/Met 336 (ERβ), and Met421 (ERα)/Ile373 (ERβ) interact with cowpea isoflavones. Effective ligands for the estrogen receptor protein should demonstrate strong interactions with Arg394/34 and Glu353/305, which are hydrogen bonds of ERα. In addition, to validate the estrogenic effect of cowpea, an in vivo test was conducted on a menopausal animal model that had been subjected to a two-week treatment with cowpea KT4, which contained a greater quantity of genistein than cowpea KT6.

Estrogenic effect of cowpea KT4 ethanolic extract

Previous research has documented that the administration of E2 mitigates structural damage to vaginal cells and cell atrophy has been identified as a factor in postmenopausal women experiencing vaginal dryness and sexual dysfunction.[24,25,26] Table 4 and Figure 4 present the results of this research, which demonstrate the development of estrogen receptors in animal models of vaginal epithelium following administration of cowpea ethanolic extract. According to the results of this research, ovariectomy induces a decrease in the expression of estrogen receptors, specifically ERα and ERβ, when utilized as a model for menopause. The administration of cowpea ethanolic extract resulted in an observed increase in the expression of ERα or ERβ, with the most significant effect observed at a dosage of 5 mg/kg BW. The administration of cowpea ethanolic extract at a dosage of 5mg/kgBW exhibited comparable efficacy to estrogen therapy at a dosage of 0.18µ mg/kgBW.

An absence of ovarian function leads to a severe deterioration of estrogen receptors in vaginal epithelium tissue. The statistical analysis of the staining of estrogen receptors that were positive in the vaginal section of the animal model confirmed that, relative to the non-treatment group, cowpea promotes an overall ER enhancement of the vaginal epithelium. A prior investigation found that the administration of Genistein led to the subsequent outcomes: reduction of vaginal tissue atrophy, reduction in vaginal pH, enhancement of vaginal index and vaginal health score, and upregulation of epidermal growth factor (EGF) and E-cadherin.[27] An additional significant study revealed that EGF, through a network of signaling pathways, promotes cell growth and maturation, facilitates the replacement of senescent cells, and accelerates collagen synthesis in compromised areas.[28]

A previous study reported that improving the vaginal stratified epithelium's thickness and restoring the vaginal environment to its premenopausal state of health is the principal aim in the management of vulvovaginal atrophy during menopause.[29]

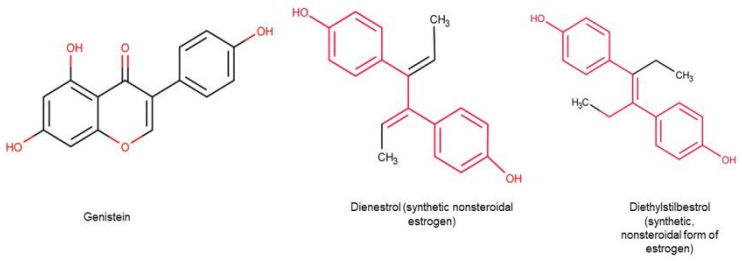

Figure 2: Structure Similarity genistein of cowpea type 4 with Drug Database

Table 2: Docking report for bioactive compounds of Cowpea KT4 with Estrogen receptor

Receptor	Ligand	CID	Binding affinity (kcal /mol)
ESR A	Estradiol	5757	-10.34
(PDB ID 1X7R)	Glycitein	5317750	-9.06
	Genistein	5280961	-9.03
	Daidzein	5281708	-8.85
	Estradiol	5757	-10.85
ESR B	Glycitein	5317750	-8.85
(PDB ID 5TOA)	Daidzein	5281708	-8.85
	Genistein	5280961	-8.85

CID=chemically induced dimerization, ESR A=estrogen receptor A, ESR B= estrogen receptor B, kcal/mol=kilocalorie per mole

Table 3: Representation of protein interaction based on isoflavone type of cowpea KT4

No	Complex	Binding affinity (kcal/mol)	Amino acid interaction
Receptor ESR A- Ligan			
1	Estradiol (Control)	-10.34	LEU384, HIS524, GLU353
2	Glycitein	-9.06	LEU384, MET421, HIS524, GLU353,
3	Genistein	-9.03	LEU384, GLU353, HIS524
4	Daidzein	-8.85	LEU384, GLU353, HIS524
Receptor ESR B- Ligan			
1	Estradiol (Control)	-10.85	MET336,HIS475,GLU305, ARG364
2	Genistein	-8.85	MET336,GLU305
3	Glycitein	-8.85	MET336,GLU305,ARG346
4	Daidzein	-8.85	MET336, ARG346

Table 4: Differences in estrogen receptor expression in animal models based on treatment Cowpea ethanolic extract (KT4)

Animal groups	ER*α*		ER*β*	
	Mean+SD	*p*	Mean+SD	*p*
G1 (placebo-operated)	8.00 ± 2.00	0.449	5.83 ± 1.47	<0.001*
G2 (Ovarektomi)	3.33 ± 1.03		3.17 ± 1.72	
G3 (Ovarektomi + Cowpea T4 dosis 0. mg/kgBB)	6.00 ± 1.21		9.67 ± 1.63	
G4 (Ovarektomi + Cowpea T4 dosis 2.5 mg/kgBB)	6.33 ± 1.51		10.83 ± 1.94	
G5 (Ovarektomi + Cowpea T4 dosis 5 mg/kgBB)	6.67 ± 1.75		12.33 ± 1.97	
G6 (Ovarektomi + E2 0.18µ mg/kgBB)	6.67 ± 2.28		12.00 ± 1.90	

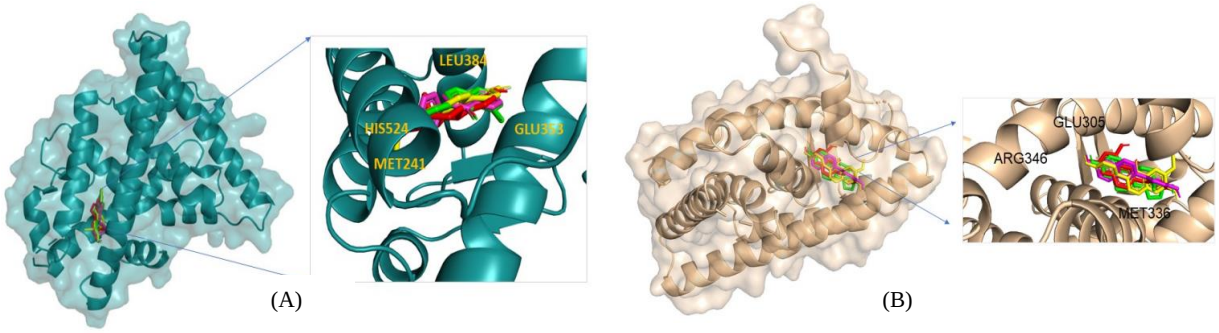

Figure 3: (A) Visualization of protein complex interaction of estrogen receptor, ER*α* with cowpea bioactive. Note Estradiol (Red), Glycitein (Yellow), Daidzein (Pink), Genistein (Green). ER*β* with cowpea bioactive

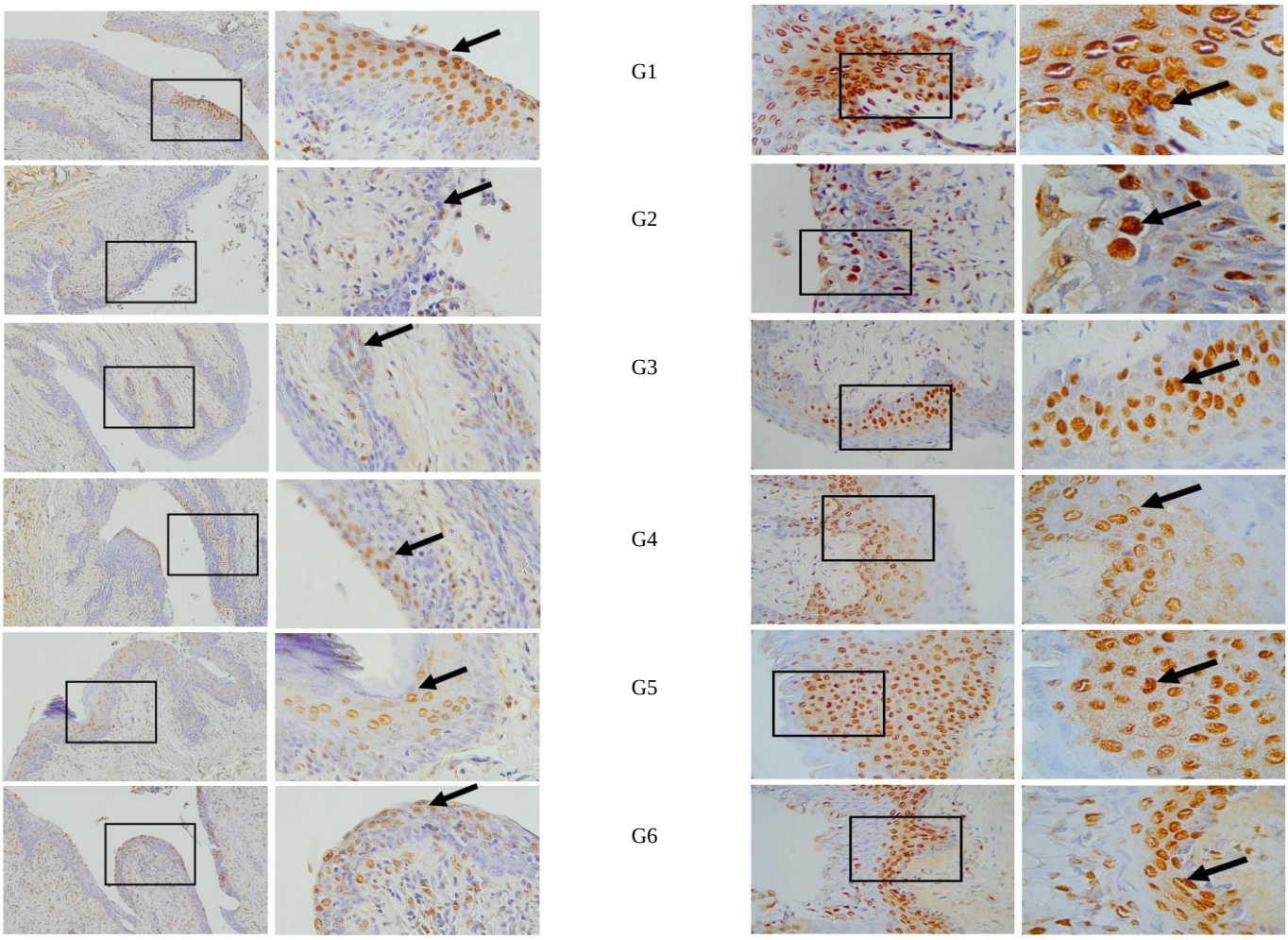

Figure 4. Profile of ERα (A) and ERβ (B) on vaginal tissue based on treated group models. Results presented in magnifications of 200x and 400x, it appears that vaginal epithelial cells expressing ERα and ERβ are marked with brown color in the cell nucleus (arrow).

Previous studies have evaluated the estrogenic properties of various isoflavones, including genistein 0.084, estradiol (E2) 100, daidzein 0.013, and S-equol 0.061,[30] from which the present study has demonstrated that cowpea, which contains isoflavones, particularly genistein, effectively promotes the enhancement of estrogen receptor expression in the vaginal epithelial tissue of menopausal animals. The efficacy of isoflavone administration in the treatment of vaginal atrophy and menopausal symptoms has been substantiated by clinical data gained from numerous studies. Subsequently, its capacity to impede the nuclear translocation of nuclear factor kappa beta (NFkB) induced by tumor necrosis factor-alpha (TNF-α) has been uncovered through molecular mechanism investigation involving genistein. [31,32] Additionally, genistein has been found to regulate cell proliferation through estrogen-dependent pathways that involve the activation of extracellular signal-regulated kinase (ERK1/2).[33,34,35,36] Further study is required to identify the estrogenic agonist in cowpea that influences genes related to vaginal epithelial cell repair, which could help explain the increased expression of CGRP, VEGF, and NGF in vaginal tissue.

Conclusion

In conclusion, variety 4 had a greater genistein content than variety 6, and it interacted at a molecular level with multiple proteins associated with estrogen signaling. Research findings confirmed the estrogenic impact of cowpea KT4 through the quantification of elevated expression of estrogen receptors, ERα and ERβ, in vaginal tissue epithelial cells within a menopausal model. Potentially, this mechanism would explore the potential use of cowpeas as a natural remedy for menopausal women, aiming to minimize the risks associated with prolonged hormonal therapy.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The authors [of this study] would like to acknowledge Mr. Kaliawan for his technical assistance in LC-MS/MS analysis.

References

1. Ambikairajah A, Walsh E, Cherbuin N. A review of menopause nomenclature. Reprod Health. 2022; 19(1):1-15.
2. Bleibel B, Nguyen H. Vaginal Atrophy [Internet]. StatPearls Publishing; 2023 [cited 2024 Mar 10]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK559297/
3. Li S, Herrera GG, Tam KK, Lizarraga JS, Beedle M-T, Winuthayanon W. Estrogen action in the epithelial cells of the mouse vagina regulates neutrophil infiltration and vaginal tissue integrity. Sci Rep. 2018; 8(1):1-13.
4. Liu ZM, Zhang D, Li G, Ho SC, Chen YM, Ma J, Huang Q, Li S, Ling WH. The 6-month effect of whole soy and purified isoflavones daidzein on thyroid function—A double-blind, randomized, placebo-controlled trial among Chinese equol-producing postmenopausal women. Phytother Res. 2021; 35(10): 5838-46.
5. Cho L, Kaunitz AM, Faubion SS, Hayes SN, Lau ES, Pristera N, Scott N, Shifren JL, Shufelt CL, Stuenkel CA, Lindley KJ. Rethinking menopausal hormone therapy: For whom, what, when, and how long? Circulation. 2023; 147(7): 597-610.
6. Hodis HN, Mack WJ. Menopausal hormone replacement therapy and reduction of all-cause mortality and cardiovascular disease: It is about time and timing. Cancer J. 2022; 28(3): 208-23.
7. Rasheed S, Rehman K, Shahid M, Suhail S, Akash MSH. Therapeutic potentials of genistein: New insights and perspectives. J Food Biochem. 2022; 46(9): 1-13.
8. Nikolić Lj I, Savić-Gajić M I, Tačić D A, Savić M I. Classification and biological activity of phytoestrogens: A review. Adv Technol. 2017; 6(2): 96-106.
9. Sureda A, Sanches Silva A, Sánchez-Machado DI, López-Cervantes J, Daglia M, Nabavi SF, Nabavi SM. Hypotensive effects of genistein: From chemistry to medicine. Chem Biol Interact. 2017; 268: 37-46.
10. Li M, Yu Y, Xue K, Li J, Son G, Wang J, Qian W, Wang S, Zheng Z, Yang C, Ge J. Genistein mitigates senescence of bone marrow mesenchymal stem cells via ERRα-mediated mitochondrial biogenesis and mitophagy in ovariectomized rats. Redox Biol. 2023; 61(102649): 1-15.
11. Sharifi-Rad J, Quispe C, Imran M, Rauf A, Nadeem M, Gondal TA, Ahmad B, Atif M, Mubarak MS,Sytar O, Zhilina OM, Garsiya ER, Smeriglio A, Trombetta D, Pons DG, Matorell M, Cardoso SM, Razis AFA, Sunusi U,Kamal RM, Rotariu LS, Butnariu M, Docea AO, Calina D. Genistein: An integrative overview of its mode of action, pharmacological properties, and health benefits. Oxid Med Cell Longev. 2021; 2021: 1-36.
12. Fithri An, Yueniwati Y, Arsana I.W, Khotimah.H, Nurwidyaningtyas.W. Targeted proteins for vaginal epithelial repair. J Pharm Pharmacogn Res 2023; 11(1): 110-6.
13. Pourhadi N, Mørch LS, Holm EA, Torp-Pedersen C, Meaidi A. Menopausal hormone therapy and dementia: nationwide, nested case-control study. Brit Med J. 2023; 381: 1-15.
14. Lega IC, Fine A, Antoniades ML, Jacobson M. A pragmatic approach to the management of menopause. Can Med Assoc J. 2023; 195(19):E677-82.
15. Andrea R Genazzani, Patrizia Monteleone, Andrea Giannini, Tommaso Simoncini. Hormone therapy in the postmenopausal years: considering benefits and risks in clinical practice. Hum Reprod Update. 2021; 27(6): 1111-1115.
16. Gonçalves A, Goufo P, Barros A, Domínguez-Perles R, Trindade H, Rosa EAS, Ferreira L, Rodrigues M. Cowpea (*Vigna unguiculata L. Walp*), a renewed multipurpose crop for a more sustainable agri-food system: nutritional advantages and constraints. J Sci Food Agric. 2016; 96(9): 2941-51.
17. Lonardi S, Muñoz-Amatriaín M, Liang Q, Shu S, Wanamaker SI, Lo S, Tanskanen J, Schulman AH, Zhu T, Luo CM, Alhakami H, Ounit R, Hasan MA, Verdier J, Roberts PA, Santoso JRP, Ndeve A, Dolezel Z, Vrana J, Hokin SA, Farmer AD, Cannon SB, Close TJ. The genome of cowpea (*Vigna unguiculata [L.] Walp.*). Plant J. 2019; 98(5): 767-82.
18. Martins TF, Souza PFN, Alves MS, Silva FDA, Arantes MR, Vasconcelos IM, Oliveira JTA. Identification, characterization, and expression analysis of cowpea (*Vigna unguiculata [L.] Walp*.) miRNAs in response to cowpea severe mosaic virus (CPSMV) challenge. Plant Cell Rep. 2020; 39(8): 1061-78.
19. Souza VR, Mendes E, Casaro M, Antiorio ATFB, Oliveira FA, Ferreira CM. Description of ovariectomy protocol in mice. In: Methods in Molecular Biology. New York, NY: Springer New York; 2019. 303-309 p.

20. Adel M, Sadegh AB, Arizza V, Abbasi H, Inguglia L, Saravi HN. Anesthetic efficacy of ketamine-diazepam, ketamine-xylazine, and ketamine-acepromazine in Caspian Pond turtles (*Mauremys caspica*). Indian J Pharmacol. 2017; 4 9(1): 93-97.
21. Křížová L, Dadáková K, Kašparovská J, Kašparovský T. Isoflavones. Molecules. 2019; 24(6):1076-1089.
22. Du Z-R, Feng X-Q, Li N, Qu J-X, Feng L, Chen L, Chen Weng-Fang. G protein-coupled estrogen receptor is involved in the anti-inflammatory effects of genistein in microglia. Phytomedicine. 2018; 43:11-20.
23. Yoriki K, Mori T, Aoyama K, Tarumi Y, Kataoka H, Kokabu T, Kitawaki J. Genistein induces long-term expression of progesterone receptor regardless of estrogen receptor status and improves the prognosis of endometrial cancer patients. Sci Rep. 2022; 12(1): 1-18.
24. Agarwal S, Alzahrani F, Ahmed A. Hormone Replacement Therapy: Would it be Possible to Replicate a Functional Ovary? Int J Mol Sci. 2018; 19(10): 3160-3172.
25. Paciuc J. Hormone Therapy in Menopause. In: Advances in Experimental Medicine and Biology. Cham: Springer International Publishing; 2020; 89-120 p.
26. Vigneswaran K, Hamoda H. Hormone replacement therapy – Current recommendations. Best Pract Res Clin Obstet Gynaecol. 2022; 81:8-21.
27. Sun Y, Wang L, Wang B, Meng Y, Wang W. Genistein up-regulates the expression of EGF and E-cadherin in the treatment of senile vaginitis. Molecules. 2022; 27(8): 1-18.
28. Ding C, Zou Q, Wang F, Wu H, Chen R, Lv J, Ling M, Wang W, Li H, Huang B. Human amniotic mesenchymal stem cells improve ovarian function in natural aging through secreting hepatocyte growth factor and epidermal growth factor. Stem Cell Res Ther. 2018; 9(1): 1-11.
29. Naumova I, Castelo-Branco C. Current treatment options for postmenopausal vaginal atrophy. Int J Womens Health. 2018; 10: 387-95.
30. Chen L-R, Chen K-H. Utilization of isoflavones in soybeans for women with menopausal syndrome: An overview. Int J Mol Sci. 2021; 22(6): 3212-3225.
31. Ghazanfarpour M, Sadeghi R, Roudsari RL. The application of soy isoflavones for subjective symptoms and objective signs of vaginal atrophy in menopause: A systematic review of randomised controlled trials. J Obstet Gynaecol. 2016; 36(2):160-71.
32. Dizavandi FR, Ghazanfarpour M, Roozbeh N, Kargarfard L, Khadivzadeh T, Dashti S. An overview of the phytoestrogen effect on vaginal health and dyspareunia in peri- and post-menopausal women. Post Reprod Health. 2019; 25(1): 11-20.
33. Khuleshwari K, Vijay P. Genistein enhances expression of extracellular regulated kinases (ERK) 1/2, and learning and memory of mouse. IBRO Neurosci Rep. 2021; 10: 90-95.
34. Smolińska E, Moskot M, Jakóbkiewicz-Banecka J, Węgrzyn G, Banecki B, Szczerkowska-Dobosz A, Bohdan DP, Ciminska GM. Molecular action of isoflavone genistein in the human epithelial cell line HaCaT. PLoS One. 2018; 13(2): 1-27.
35. Ishola AO, Ogundola FA, Liasu OM, Sunday OA, Adeeyo OA. View of the effect of inoculation with Glomus mosseae on early growth and pattern of root exudates in potted Cowpea [*Vigna unguiculata (L.) Walp*]. Trop J. Nat Prod Res. 2022; 6(1): 76-79.
36. Oboh G, Akerele GP, Adedayo BC, Ademiluyi AO. Comparative studies of the effects of five indigenous Cowpea (*Vigna unguiculata*) varieties on enzymes linked to type 2 diabetes and their glycemic indices. Trop J. Nat Prod Res. 2021; 970-976.