Asian Journal of Health Research
Journal Homepage: https://a-jhr.com
Published by Ikatan Dokter Indonesia Wilayah Jawa Timur

Original Research

The Effect of Isoflavone in Cowpea (Vigna unguiculata) Powder Supplement on Post-Menopausal Vaginal Maturation Index at Malang, Indonesia

Tatik Sujiati, Budi Siswanto, A Hidayat and Ayu Rizky Widowati*
Department of Obstetrics and Gynecology, Faculty of Medicine, Brawijaya University/Saiful Anwar General Hospital, Malang, East Java, Indonesia

ARTICLE HISTORY
Received: 22 January 2022
Revised: 10 February 2022
Accepted: 17 February 2022

CORRESPONDING AUTHOR
Ayu Rizky Widowati
ayurzky19@gmail.com
Department of Obstetrics and Gynecology, Faculty of Medicine, Brawijaya University/Saiful Anwar General Hospital, Malang, East Java, Indonesia

KEYWORD
Isoflavone; Cowpea; Maturation vaginal index.

ABSTRACT

Introduction: Only a few published studies have directly demonstrated the effects of local soy isoflavone on the post-menopausal vaginal maturation index. This study focused on the effect of 24 weeks of local soy isoflavone (67.5 mg) consumption to improve the post-menopausal vaginal maturation index.

Methods: 50 post-menopausal women were randomly assigned to a double-blind clinical trial to receive cowpea (Vigna unguiculata) powder treatment (n=25) and placebo (n=23). Vaginal cytology (maturation index, maturation value) was evaluated at pre and post-treatment in Anatomy Pathology Laboratories. Collected data were analyzed by using the analysis of covariant (ANOVA) method.

Results: There is no difference in maturation vaginal index between the treatment and the placebo group. The treatment was not significantly improved the vaginal maturation index ($p<0.05$). The concomitant variable, age, post-menopausal interval, BMI, marital status, and initial cell condition have no significant effect on the maturation vagina index.

Conclusion: Cowpea powder supplement with 67.5 mg isoflavone cannot improve the vaginal maturation index on post-menopausal patients.

Cite this as: Sujiati T, Siswanto B, Hidayat A, Widowati AR (2022) The Effect of Isoflavone in Cowpea (Vigna unguiculata) Powder Supplement on Post-Menopausal Vaginal Maturation Index at Malang, Indonesia. *Asian J Heal Res* 1:12–17. doi: https://doi.org/10.55561/ajhr.v1i1.10

INTRODUCTION

The decrease in estrogen at menopause can cause women to experience various short-term and long-term complaints. In the short term, the decline in estrogen levels in the peri and post-menopausal periods cause a group of symptoms called a climacteric syndrome. The most prominent symptoms are vasomotor symptoms, followed by psychological and psychosocial symptoms. In the long term, decreased estrogen levels can increase the risk of cardiovascular disease, osteoporosis, Alzheimer's dementia, genital atrophy, and degradation changes in non-genital organs [1-3].

Reduced estrogen causes various changes in the reproductive organs. Genital organs such as the uterus and vagina undergo atrophy. Vaginal atrophy occurs on average 4-5 years after menopause. Vaginal atrophic changes include reduced length and diameter, characteristic pale pink coloration due to reduced mucus production by superficial glands, minimal glycogen production, inhibited lactobacilli growth, increased pH, and overgrowth Streptococcus, Staphylococcus, and Coliform bacilli. The current therapy is hormone replacement therapy (TSH). However, TSH has several disadvantages, such as high cost, unwanted side effects, and an increased risk of malignancy in specific populations that limit its use [2,4-6].

Many have published a compound similar to estrogen found in plants. These compounds are known as phytoestrogens with the dynamic content of isoflavones. It is known that foods containing isoflavones are beneficial for menopausal health and can

even complement TSH because of their structure and function that resembles estrogen [7,8]. This study discusses the clinical trial of cowpea powder (Vigna Unguiculata) which mostly comes from Malang, on improving post-menopausal vaginal epithelial growth.

MATERIAL AND METHODS

Study Design

This study was experimental research using a double-blind, randomized clinical trial. This study undergoes seven months with 50 participants. Inclusion criteria were menopausal married women and agreed to follow the experiment protocol. Exclusion criteria were subjects with diabetes mellitus (fasting blood sugar > 140 mg/dL), women with hormone replacement therapy, women in other hormonal therapy, using tetracycline topical in 1 week before the examination, digitalis usage in 2 years before the examination, undergo radiotherapy or cauterization vagina in the last six weeks, and corticosteroid usage in 3 weeks before the examination.

Experimental Procedures

All participants who fulfill inclusion criteria signed the informed consent and were divided into two groups. Group A was given cowpea with 67,5 mg isoflavone with skim milk carrier once a week. Group B was given a placebo with a skim milk carrier once weekly. Cowpea extraction was made in yogurt and packed with a sachet for 24-weeks of administration. Vagina cytology was performed two times with a 3-weeks interval in the intervention period.

Ethics

All techniques in this study were carried out in compliance with the appropriate manuals and regulations and were approved by the Health Research Ethics Committee, Faculty of Medicine, Brawijaya University, Malang, Indonesia.

Statistical Analysis

All data were evaluated using SPSS 11.0 for Windows software. One-way analysis of covariance (ANOVA). P<0.05 was considered to indicate statistical significance. Data represent mean ± standard deviation (SD).

RESULT

Subject Characteristic

From 50 participants, only 48 participants can be evaluated due to 2 patients moving outside the town, divided as 25 in group A and 23 in group B. The baseline characteristic of the patients is served in Table 1.

Table 1. Baseline characteristic of the subjects

Variable	Mean ± SD
Age	50,50 ± 6,776
Menopausal Age	48,31 ± 3,685
Menopause duration	12,190 ± 8,076
Body Mass Index (BMI)	23,880 ± 4,030
First parabasal cell	43,44 ± 43,810
First Intermediate cell	44,48 ± 36,467
First Superficial cell	12,080 ± 17,875
Beginning maturation volume	34,323 ± 28,056

Effect of Treatment on Parabasal Cells

The results of the comparative analysis of treatment and control of parabasal cells after three months of treatment obtained p-value = 0.855. BMI and marital status were non-significant confounding variables (p-values 0.588 and 0.110, respectively), while the initial parabasal cell confounding effect was significant with a p-value = 0.001. On the other hand, the variables of age, age of menopause, and duration of menopause cannot be calculated, so interpretation cannot be made.

The results of the comparative analysis of the group and control of parabasal cells after six months of treatment obtained p-value = 0.688. BMI and marital status were non-significant confounding variables (P values were 0.821 and 0.217, respectively), while the initial parabasal cell confounding effect was significant with P=0.016. On the other hand, the variables of age, age of menopause, and duration of menopause cannot be calculated, so interpretation cannot be done.

Effect of Treatment on Intermediate Cell

The results of the comparative analysis of treatment and control of parabasal cells after three months of treatment obtained p-value = 0.981. BMI and marital status were non-significant confounding variables (p-values 0.886 and 0.059, respectively). On the other hand, the variables of age, age of menopause, and duration of menopause cannot be calculated, so interpretation cannot be made.

The results of the comparative analysis of the group and control of parabasal cells after six months of treatment obtained p-value = 0.936. BMI and marital status were non-significant confounding variables (P values were 0.959 and 0.149, respectively), while the initial parabasal cell confounding effect was significant with P=0.006. On the other hand, the variables of age,

Table 2. Mean value of Parabasal cell, intermediate cell, superficial cell, and maturation volume

Condition	N	Mean	Standard Deviation
Parabasal Cell in 3 months			
Control	23	71.91	40.65
Treatment	25	66.00	37.91
Parabasal Cell in 6 months			
Control	23	69.39	41.12
Treatment	25	63.44	41.73
Intermediate cell in 3 months			
Control	23	25.74	37.01
Treatment	25	31.24	36.68
Intermediet Cell in 6 months			
Control	23	28.52	38.62
Treatment	25	32.04	37.35
Superficial cell in 3 months			
Control	23	2.35	5.43
Treatment	25	3.04	7.23
Superficial Cell in 6 months			
Control	23	1.91	3.59
Treatment	25	4.52	8.91
Maturation Volume in 3 months			
Control	23	45.22	22.33
Treatment	25	18.66	20.08
Maturation Volume in 6 months			
Control	23	16.1739	21.6205
Treatment	25	20.5400	23.6982

Table 3. Mean value of MV during treatment

Condition	Starting	3 months	6 months
Control	34.32	15.2174 (44.34%)	16.1739 (47.13%)
Treatment	34.32	18.6600 (54.37%)	20.5400 (59.85%)

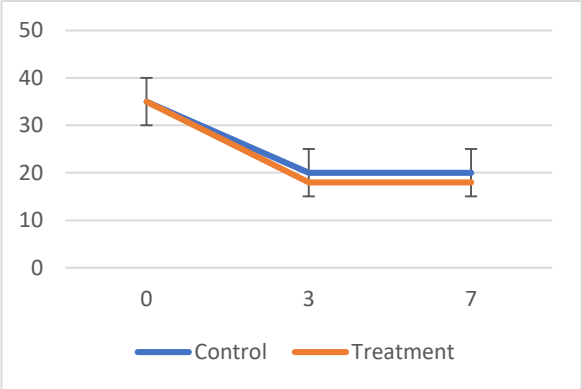

Fig. 1. Effect of Cowpea Powder on Vaginal Epithelial (MV) Growth

age of menopause, and duration of menopause cannot be calculated, so interpretation cannot be done.

Effect of treatment on Maturation Volume

The comparative analysis of treatment and control results on maturation volume (MV) after three months of treatment obtained p-value = 0.835. BMI and marital status were non-significant confounding variables (p-values 0.395 and 0.200, respectively), while the initial maturation volume effect as a confounder was significant with p=0.001. On the other hand, the variables of age, age of menopause, and duration of menopause cannot be calculated, so interpretation cannot be made.

The results of the comparative analysis of the control group and the maturation volume after six months of treatment obtained p-value = 0.860. BMI and marital status were non-significant confounding variables (P values were 0.645 and 0.318, respectively), while the initial maturation volume effect as a confounder was not significant with P-value = 0.053. On the other hand, the variables of age, age of menopause, and duration of

menopause cannot be calculated, so interpretation cannot be made.

The mean value of the parabasal cell, intermediate cell, superficial cell, and maturation volume are described in Table 2. The mean value of MV pre and post-treatment are described in Table 3 and Fig. 1.

DISCUSSIONS

At the end of the treatment, only 48 people could be analyzed in the control group, and research subjects were excluded due to domicile change. The level of acceptance of research subjects on research materials is quite good. This can be seen from the subjects excluded not because of the materials consumed. The average length of menopause in the subjects of this study was 12.19 years, with the average age of the study subjects being 60.50 years. Most subjects were not obese, with an average BMI of 23.88 years.

In this study, BMI did not affect vaginal epithelial growth. These data are the same as Alison et al. (1999). This findings is certainly not in accordance with the literature review, which explains a relationship between body fat and extra glandular production, which was previously relatively small compared to estrogen from the ovaries. An increase in blood estrone levels characterizes the increased role of extra glandular estrogen. This increase in estrone is due to the ability of body tissues outside the ovaries to convert androstenedione and testosterone into estrone. This conversion is possible because estrone is formed from androstenedione and testosterone with the aromatase enzyme as a catalyst in the steroid hormone biosynthesis pathway. In this case, fat tissue has the most remarkable ability to convert androstenedione into estrone. Although the biologic effect is not as strong as that of estradiol, in conditions of ovarian insufficiency, this estrone level is quite significant in compensating for estradiol deficiency [9,10].

Marital status also did not significantly affect the growth of the vaginal epithelium. In several studies, a significant relationship between marital status and changes in the vaginal epithelium maturation index appears to be only a statistically significant relationship. No theory or data is showing the relationship between the two until now. According to Bacfhman, couples who are still sexually active show a more elastic vagina, and with the natural lubrication of the sex response, they complain less of vaginal atrophy. His research showed a positive correlation between sexual activity with gonadotropins and androgens, both of which are essential components for vaginal epithelial health when endogenous estrogen levels decrease [11,12].

In this study, the administration of cowpea powder containing 67.5 mg of isoflavones did not significantly affect the growth of vaginal epithelium (MV). This is different from the results of previous studies, which showed that isoflavone phytoestrogens help increase vaginal epithelial cell growth, including the study of Wilcox et al. (1990), who first showed that a diet rich in isoflavone phytoestrogens (soy, flour, red clover, flaxseed) could increase vaginal epithelial growth (MV). Murkis (1995), by giving 200 cc of soy milk to post-menopausal women for 12 weeks, also showed an increase in the vaginal maturation index. Baird (1995) studied 97 post-menopausal women given isoflavones 165 mg/day and found an increase in vaginal maturation index compared to placebo, but this difference was not statistically significant [13].

According to the literature review, estrogen receptors can be demonstrated by immunohistochemistry and dent radiographic methods that estrogen receptors are present in the epithelium, stroma, and muscle fibers of the vagina. According to Achmad Biben, the bonding of isoflavones to the estrogen receptor goes through a process similar to that of the natural estrogen hormone. Estrogen is flowed through blood vessels to reach cells and occupy estrogen receptors. Hormones bind to these receptors to form hormone-ligand receptor complexes that stimulate cell-specific genes [14,15].

Table 4 and figure 1 show that after six months of treatment, the effect did not show statistical significance, and overall vaginal epithelium was still categorized as atrophic (MV < 50). This study is consistent with the literature that given the lower affinity of isoflavones than estradiol in binding to the estrogen receptor, the result is not as strong as the mechanism of action of endogenous estradiol on target cells. Isoflavones have natural SERM properties, can bind to both estrogen receptor isoforms, namely RE-α and RE-β with 8-40 times stronger on RE-β [16].

Chen et al. reported that isoflavones have little effect on the vaginal wall. In premenopausal women, there are estrogen receptors α and β in the vaginal wall. However, after menopause, almost all estrogen β receptors are lost. The decrease in phytoestrogens for clinical purposes, especially vaginal atrophy, is still not widely known. Although there are many reports regarding the benefits of dietary phytoestrogens on the health of post-menopausal women, the molecular mechanisms in cells are not fully understood. Further research on phytoestrogens is still needed [17].

This research finding is in accordance with the findings of a systematic review published in 2017 by Saghafi et al.18, which show that soybeans and phytoestrogens have no statistically significant favorable impact on vaginal atrophy score. Phytoestrogens have varying effects on vaginal atrophy depending on the route and kind of administration. Topical isoflavone therapy may be effective in the treatment of vaginal symptoms.19 Topical application of an isoflavone-

containing gel may help resolve vaginal dryness and maturation value 20. As a result, it is proposed that in situations with non-severe vaginal atrophy, non-hormonal therapies and other treatments such as vaginal gels should be used more often [18].

Cowpea (V. unguiculata) is a source of various isoflavones with antioxidant and anti-inflammatory properties, such as genistein, quercetin, and daidzein. Isoflavones have a molecular structure similar to that of 17β-estradiol and also have estrogenic activity. These compounds bind directly to the same receptors as endogenous estrogens, resulting in estrogenic effects [19]. Ozacmak and Sayan reported that treatment with 17β-estradiol and progesterone reduced the MDA levels in the brains of ovariectomized rats. In addition, estrogen suppresses the formation of free radicals because treatment with 17β-estradiol lowers MDA levels in the brain of ovariectomized rats [20].

Both ERα and ERβ can bind to isoflavones, and ERβ has a stronger affinity. Therefore, ERβ plays a central role in the ovarian system, cardiovascular system and brain. Another mechanism of isoflavone acts as a hydrogen donor from phenol groups to peroxide radicals. The radical phenoxyl stability resulting from the reaction prevents the propagation of lipid peroxidation. Therefore, free radical reactivity can be controlled and the free radical antioxidants formed from the reaction are reduced which promote MV growth [21].

This research has several weaknesses. The side effects of giving cowpea powder containing 67.5 mg of isoflavones, especially on the uterus and breasts, have not been evaluated due to limited funds and available time. There has been no study on other ingredients in cowpea powder that might affect the growth of vaginal epithelium. There is a long-time difference between the results of the vaginal cytology examination before treatment and the start of the intervention, which is about 5 months. During this time, there may have been changes in the growth of the vaginal epithelium in the study subjects. This is because this research is a follow-up study from previous research.

CONCLUSION

The comparative analysis results in this study concluded that the administration of cowpea powder containing 67.5 mg of isoflavones could not improve the growth of post-menopausal vaginal epithelium. For better research, it is recommended to control confounding factors more by selecting samples with more homogeneous confounding factors, especially BMI and marital status. Multicenter research is needed with a larger sample size, a larger dose, and a longer time to ensure the accuracy of the research results.

ACKNOWLEDGMENTS

We thank all those who had supported this study and helped collect data.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

REFERENCES

1. Hanafiah M. Gambaran umum menopause, definisi, demografi, dan epidemiologi. Presented in Basic Menopausal Course PIT XIII POGI Malang, 2002.
2. Gomez-Zorita S, Gonzales-Arceo M, Fernandez-Quintela A, Eseberri I, Trepiana J, Portillo MP. Scientific Evidence Supporting the Beneficial Effects of Isoflavones on Human Health. *Nutrients* 2020, 12, 3853; doi:10.3390/nu12123853
3. Imhof M, Gocan A, Schmidt M. Soy germ extract alleviates menopausal hot flushes: placebo-controlled double-blind trial. *European Journal of Clinical Nutrition* volume 72, p.p 961–970, 2018.
4. Baziad A. SIklus Menstruasi, Perimenopause, Menopause dan Pasca Menopause. Presented in Basic Menopausal Course PIT XIII POGI Malang, 2002.
5. Ahsan M, Mallick AK. The Effect of Soy Isoflavones on the Menopause Rating Scale Scoring in Perimenopausal and Postmenopausal Women: A Pilot Study. *J Clin Diagn Res*. 2017 Sep; 11(9): FC13–FC16
6. Speroff L, Glass Rit, Kase NG. Menopause and the perimenopause transitition. In: Clinical gynecology endocrinology and infertility, editor by Mitchell C, 9th edition, Baltimore, Lippincot William & Wilkin, 2011;pp. 643-667.
7. Liu Y, Li J, Wang T, Wang Y, Zhao L, Fang Y. The effect of genistein on glucose control and insulin sensitivity in postmenopausal women: a meta-analysis. *Maturity*. 2017; 97:44–52. PMID: 28159061.
8. Mazambani S, Johnson K, Vemuri S, Alshafi S, Cheriyath V. Daidzin-rich Soy Isoflavone Extracts Promote Estrous Cycling in VCD- induced Menopause Mouse Model. Nutri Food Sci Int J 4(4): NFSIJ.MS.ID.555644, 2018.
9. Maliehe A, Ghahremani S, Kharghani S, Ghazanfarpour M, Shariati K, Kazemi M, *et al*. Effect of Isoflavones and Genistein on Glucose Metabolism in Peri- and Post-Menopausal Women: An Overview of Meta-Analysis. *Journal of Menopausal Medicine* 2019; 25(2): 69-73.
10. César Velasco-Téllez, Manuel Cortés-Bonilla, Guillermo Ortiz-Luna, Linda Sánchez-Zelayeta, Horacio Méndez-Serrano, Cinthya Salazar-Jiménez, Abraham Zavala-García and Alicia Sánchez-Cevallos (October 7th 2020). Quality of Life and Menopause, Quality of Life - Biopsychosocial Perspectives, Floriana Irtelli,

Federico Durbano and Simon George Taukeni, IntechOpen, DOI: 10.5772/intechopen.88983. Available from: https://www.intechopen.com/chapters/69559

11. EFSA. Risk assessment for peri- and postmenopausal women taking food supplements containing isolated isoflavones. EFSA J. 2015;13:4246.
12. Messina M. Soy and health update: evaluation of the clinical and epidemiologic literature. Nutrients. 2016;8:754.
13. Li L, Lv Y, Xu L, Zheng Q. Quantitative efficacy of soy isoflavones on menopausal hot flashes. Br J Clin Pharmacol. 2015;79:593–604.
14. Fang K, Dong H, Wang D, Gong J, Huang W, Lu F. Soy isoflavones and glucose metabolism in menopausal women: a systematic review and meta-analysis of randomized controlled trials. Mol Nutr Food Res. 2016; 60:1602–1614. PMID: 27004555.
15. Thomas AJ, Ismail R, Taylor-Swanson L, Cray L, Schnall JG, Mitchell ES, et al. Effects of isoflavones and amino acid therapies for hot flashes and co-occurring symptoms during the menopausal transition and early postmenopause: a systematic review. Maturitas. 2014;78:263–7.
16. Liu ZM, Ho SC, Chen YM, Tomlinson B, Ho S, To K, et al. Effect of whole soy and purified daidzein on ambulatory blood pressure and endothelial function--a 6-month double-blind, randomized controlled trial among Chinese postmenopausal women with prehypertension. *Eur J Clin Nutr*. 2015;69:1161–8.
17. McLean AC, Valenzuela N, Fai S, Bennett SA. Performing Vaginal Lavage, Crystal Violet Staining, and Vaginal Cytological Evaluation for Mouse Estrous Cycle Staging Identification. J Vis Exp; 2012 (67): e4389
18. Saghafi, N., Ghazanfarpour, M., Sadeghi, R., Hosseini Najarkolaei, A., Ghaffarian Omid, M., Azad, A., Bakhtiyari, M., & Hosseini Najarkolaei, E. (2017). Effects of Phytoestrogens in Alleviating the Menopausal Symptoms: A Systematic Review and Meta-Analysis. Iranian journal of pharmaceutical research : IJPR, 16(Suppl), 99–111.
19. Behr M, Oehlmann J, Wagner M. Estrogens in the daily diet: In vitro analysis indicates that estrogenic activity is omnipresent in foodstuff and infant formula. Food Chem Toxicol 2011; 49(10): 2681-2688.
20. Al-Baghdadi O, Ewies AAA. Topical estrogen therapy in the management of postmenopausal vaginal atrophy: An up-to date overview. J Climacteric 2009; 12(2): 91- 105.
21. Afif Z, Rahmawati D, Dalhar M, Widodo MA. Effect of ethanolic cowpea (Vigna unguiculata) extract on MDA, SOD, TNF-α levels and neuron cell in wistar rat brain after exposed with gasoline fume. IOSR J Environ Sci Toxicol Food Technol 2014; 8(2): vacuolation 113-118.