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Original Article

Phytochemical effects of soy isoflavones consumption on urinary estrogen levels in premenopausal and postmenopausal women with breast cancer

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Abstract

Breast cancer is the first of the top ten malignancies in Iraq. The benefits of consuming soy foods for hormone-positive breast cancer women are controversial. This study aimed to evaluate the effects of soy isoflavones on urinary estrogens levels and to investigate the association between urinary estrogens and urinary isoflavones in premenopausal (PreM) and postmenopausal (PostM) women with hormone-positive breast cancer treated with anti-estrogens. A randomized, interventional comparison study was carried out on 120 Iraqi women with hormonal-positive and HR2 Negative breast cancer. Participants were asked to fill out a questionnaire and to get urine samples. All participants were given (½ cup of soymilk daily) for one month and (one cup of soymilk daily) for another month. The fasted morning urine samples were collected monthly for urinary isoflavones and estrogens HPLC analysis. The mean estradiol level in PreM was significantly higher than in PostM, but after low and high doses of soymilk, it was much lower in PreM than in PostM women. While the mean estrone concentrations at low dose of soymilk were higher in PreM than in PostM women, but after high dose soymilk lower in PreM than in PostM women. Estrone level has biphasic non-significant change after isoflavone intake. Estrodail and Didazain level in PreM is lower than in PostM women after soy intake. Genstaine levels in PreM are higher than in PostM beyond soy intake. Patients who experience low urinary estradiol levels on soy food have less tendency to disease progression.

Keywords: breast cancer, urinary estradiol, urinary estrone, genistein, daidzein.

Introduction

Hormone-positive breast carcinoma is the most common type of breast cancer worldwide and in different ages. It is projected that about 70% of breast cancers are positive for estrogen receptors and about 65% are positive for progesterone receptors. About 11% of breast cancer patients are premenopausal (PreM) women. Endocrine interventions are the mainstay treatment for estrogen, progesterone, or both positive breast cancers, the anti-estrogen hormonal therapy used for a minimum of five years [1].

Isoflavones in soy foods have a structure similar to mammalian 17β-estradiol and can bind with the estrogen receptor. Soy proteins are selective estrogen receptor modulators that exert estrogen-like effects [2]. They bind to ERβ 20 times higher than binding to ERα [3].

Studies about the beneficial role of isoflavone-containing foods on different health consequences vary. Numerous studies show that soy isoflavones consumption has a role in preventing bone loss, decreasing the occurrence of hot flashes, protecting against diabetes (type 2) and protecting against breast cancer [4–8]. However, other studies revealed no effects [9–11]. This variation in the results might be related to the wide difference in the study designs, inspected populations, exposure duration to isoflavones, quantities of proteins in the products and involved products.

The beneficial role of soy foods varies according to the status of menopause, and it was proposed that the protective effect in PreM women might be greater than in postmenopausal (PostM) women [12]. At the same time, another study reported that the protective effect in PostM women was stronger than in PreM women [13]. In addition, earlier studies established that the correlation between soy isoflavone consumption and estrogen levels varies between the PreM and PostM women.

The cross-sectional research on PreM women shows inverse relationships between soy foods consumption and serum estrogen levels [13] and between free estrogen in plasma and urinary isoflavone levels [14]. Whereas interventional researches on PreM women revealed that the level of circulating estrogen was reduced by soy food consumption [15–17], while other studies revealed that the level of circulating estrogen increased by soy isoflavone foods consumption [18–20]. Furthermore, a meta-analysis study revealed that the level of circulating estrogen was not affected by soy isoflavone foods consumption [21].

Instead, there have been preliminary studies on the impact of soy isoflavone foods consumption on the level of circulating estrogen in PostM women. The previous study showed that the level of circulating estrogen was reduced [22] or unchanged [23] by soy isoflavone foods consumption in PostM women. Another study examined the correlation between soy isoflavone foods consumption and urinary level of isoflavones with the urinary levels of estrone and estradiol in PreM and PostM women and concluded that the relations of urinary levels of isoflavones with the urinary levels of estrogen varied with the menopausal status [22].

Therefore, this study aimed to investigate the effect of soy isoflavone foods consumption on the urinary isoflavones (Genistein and Daidzein), estradiol and estrone levels in the PreM and PostM women with hormone-positive breast cancer treated with anti-estrogens.

Material and Methods

Study design and setting

A randomized, interventional comparison study was carried out from October 2021 to May 2022. Almost 120 Iraqi women aged 25 to 75 years diagnosed with breast cancer hormonal positive (ER+ and PR+) and HER2 negative and treated with anti-estrogen were involved in this study. About 72 women of them were PostM and 48 women were PreM recruited to this study during their visit to the Oncological centers in Misan, south of Iraq. Women are defined as PreM when the time from the last menstruation is less than 1 year and as PostM when the time from the last menstruation is more than 1 year.

Exclusions were the high soy consumers, such as vegans and patients with metastatic breast cancers. In addition, patients with diarrhea were excluded because of malabsorption and patients taking medicines, including antibiotics such as penicillin and quinolones, were also excluded because of the suggestion that the health advantages of soy proteins are partially reliant on the microbiota of the gut.

Participants

Participants were asked to fill out a questionnaire including age, marital status, education, employment, body weight, body height, food habits, history of diseases (if there are comorbid conditions such as diabetes, hypertension, heart failure or other morbidities), drugs or herbs intake, years since menopause for PostM women, family history of BC and the ovarian suppression drug used.

All participants were asked to get fasted samples of urine specimens in the morning directly after waking up using special kits for urine collection. Urinary levels of daidzein, genistein, estradiol and estrone were measured in the Department of Pharmacology and Toxicology, Institute of Pharmacy, University of Basrah, using the high-performance liquid chromatographic technique (HPLC).

Afterward, all participants were given a low dose of soy isoflavones (½ cup of soymilk equal to 35 mg of soy isoflavones) every day for one month with daily follow-up for any unwanted signs or symptoms. After

30 days of the low dose, the fasted morning samples of urine specimens were collected and analyzed for urinary isoflavones and estrogens.

Later, all participants were given a high dose of soy isoflavones (one cup of soymilk that is equal to 70 mg of soy isoflavones) every day for one month with daily follow-up and the fasted morning samples of urine specimens collected after 30 days of the high dose and analyzed for urinary isoflavones and estrogens.

Method for preparation of urine samples

Urine (300 µl) was mixed with 200 µl of acetate buffer (pH=5.0) and 10 µl of taxifolin (10 µg/ml) as an internal standard before enzyme hydrolysis. After that, a mixture of 10 µl β-glucuronidase (5,000 U/ml ~200 U) and 10 µl of H. pomatia types H-5 and H-1 (500 U/ml ~10 U) was added and incubated for 2h at 37˚C for enzymatic hydrolysis. After incubation, 450 µl of N, N- dimethylformamide (DMF) and 40 µl formic acid were added. Samples were allowed to equilibrate for 10 min with a vortex step (30s) after 5 min and were then centrifuged (10,000 rpm × 15 min, – 3˚C) before HPLC.

HPLC analysis was performed on Dionix isocratic equipment, a Merck (Germany) Chromolith x RPC18E, 4.6 10 mm column, and a 10 µL injection used to analyze soya and hormone samples. The column oven temperature was set at 30 °C and 10 µL of sample was loaded into the HPLC injector (by Hamilton syringe, 100 µL) to start the run. This procedure is used for analyzing all different samples [24].

Quantitation was performed by UV response (254, 291, 281 and 225 nm) for daidzein, genistein, estradiol and estrone, respectively. In each group of samples, laboratory accuracy was assessed by analyzing the standard solution. The limits of detection (LOD) were 0.026nmol/ml for daidzein, genistein, estradiol and estrone.

Statistical analysis

Statistical analysis was performed using Graph Pad Prism software (version- 7.0- Inc., San Diego, CA). Descriptive statistics are measured as numbers and percentages. Mean, median, and SD for categorical data were calculated. An association between PreM and PostM women was measured using an unpaired independent t-test. One–way ANOVA analysis is used to describe the association between groups. A two-sided P value of less than 0.05 is considered statistically significant.

Table 1: Comparison between pre and postmenopausal women in patients' characteristics distribution (n=120).

Variables		Premenopausal women (n=48)*	Postmenopausal women (n=72)	t-test	P-value
Age (years)		37.91±3.476	55.2±8.12	12.996	<0.0001
Weight (kg)		73.09±12.421	80.28±17.063	2.058	0.046
Height (cm)		158.59±7.167	159.61±6.431	0.72	0.476
BSA (m^2)		1.78±0.135	1.69±0.143	3.175	0.003
Marital status	Yes	35 (79.5)	71 (98.6)	2.705	0.01
	No	9 (20.5)	1 (1.4)		
Employment	Yes	9 (20.5)	10 (13.9)	-1.159-	0.253
	No	35 (79.5)	62 (86.1)		
Comorbid	Yes	8 (18.2)	51 (70.8)	6.312	<0.0001
	No	36 (81.8)	21 (29.2)		
Past-surgical history	Yes	47 (95.5)	71 (98.6)	0.476	0.636
	No	2 (4.5)	1 (1.4)		
Family history	Yes	18 (40.9)	22 (30.6)	-1.301-	0.2
	No	26 (59.1)	50 (69.4)		
Ovarian suppression		Goserelin (Zoladex)	No need	-	-

Note: * – Four cases missed follow-up.

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RESULTS

A randomized, interventional comparison study was carried out on 120 Iraqi females with hormone-positive breast cancer aged 25 to 75 years. About 72 were PostM women with a mean age of (55.2±8.12) years and 48 were PreM women with a mean age of (37.91±3.476) years recruited to this study. The demographic characteristics of the subjects are illustrated in Table 1.

Breast cancer characteristics

Regarding the breast cancer features, there was no significant difference in the stages between the PreM and PostM participant women. Furthermore, all PostM women underwent surgical intervention, while 39 (88.6%) PreM women were exposed to surgery. Concerning chemotherapy and radiotherapy, there was no significant difference between the PreM and PostM participant women in the use of chemotherapy and radiotherapy. Regarding hormonal therapy, there is a significant difference (<0.0001) in the use of hormonal therapy, 4 (9.1%) PreM women received Anastrazole, whereas 40 (90.9%) received Tamoxifen. However, 62 (86.1%) PostM women received Anastrazole, and the rest 10 (13.9%) received Tamoxifen. The overall mean duration of hormonal therapy of PostM women was statistically higher (P=0.004) than the overall mean duration of hormonal therapy of PreM women, as illustrated in Table 2.

Urinary level of Estradiol

The results of this study revealed a significant (p<0.0001) decline in urinary estradiol levels in PreM and PostM women after one-month consumption of low dose (35 mg) and one-month consumption of high dose (70 mg) of soy isoflavones as soymilk, as demonstrated in Figure 1.

However, there is no significant difference in estradiol levels between PreM and PostM women, at baseline, after one-month consumption of a low dose (35 mg) and one-month consumption of a high dose (70 mg) of soy isoflavones, as demonstrated in Table 3.

Urinary level of Estrone

The results of this study revealed a significant (p<0.001) increase in urinary estrone level after one-month consumption of a high dose (70 mg) of soy isoflavones as soymilk. However, there is no significant difference in urinary estrone level after one-month consumption of a low dose (35 mg) of soymilk, as demonstrated in Figure 2.

Table 2: Comparison between pre and postmenopausal women according to the breast cancer characteristics.

Variables		Premenopausal women (n=48)*	Postmenopausal women (n=72)	t-test	P value
T	2	25 (56.8)	28 (38.9)	1.425	0.161
	3–4	19 (43.2)	44 (61.1)		
N	1–2	31 (70.4)	53 (73.6)	0.198	0.84
	3	13 (29.6)	19 (26.4)		
	No	44 (100)	72 (100)		
Surgery	Yes	39 (88.6)	72 (100)	2.348	0.024
	No	5 (11.4)	0		
Chemotherapy	Yes	48 (100)	68 (94.4)	-1.431-	0.16
	No	0	4 (5.6)		
Radiotherapy	Yes	41 (93.2)	63 (87.5)	-0.703-	0.486
	No	3 (6.8)	9 (12.5)		
Types of hormonal therapy	Anastrazole	4 (9.1)	62 (86.1)	-11.358-	<0.0001
	Tamoxifen	40 (90.9)	10 (13.9)		
Hormonal therapy duration (years)		2.2±0.978	3.5±2.426	3.06	0.004

Note: * – Four cases missed of follow-up.

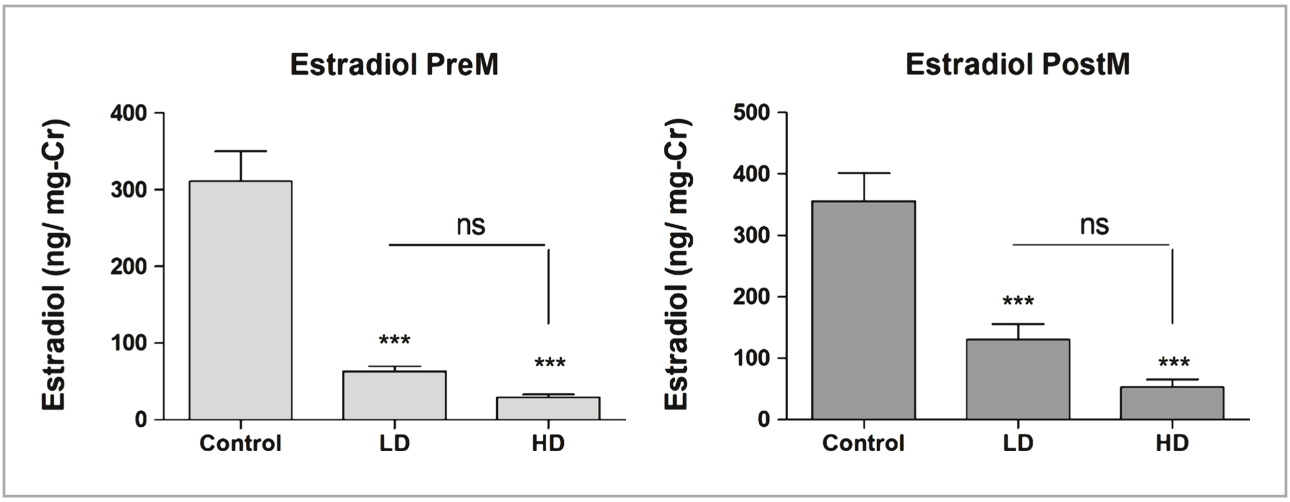

Figure 1: Urinary estradiol level according to soy isoflavone intake in premenopausal and postmenopausal women. Baseline (control), low dose (LD) 35 mg and high dose (HD) 70 mg of soy isoflavone as soymilk. Data illustrated as mean±SEM. (*** – p<0.0001; ** – p<0.001 and * – p<0.05) linked to the control.

Furthermore, there is no significant difference in urinary estrone level between PreM and PostM women, at baseline, after one-month consumption of a low dose (35 mg) and one-month consumption of a high dose (70 mg) of soy isoflavones, as demonstrated in Table 3.

Urinary level of Genistein

The results of this study revealed a significant (p<0.0001) increase in urinary genistein levels in PreM and PostM women after one-month consumption of

Table 3: Comparison between pre and postmenopausal women in urinary estradiol, estrone, genistein and diadzein levels before and after soymilk consumption.

Variables	Soymilk intake	Premenopausal women (n=48)*	Postmenopausal women (n=72)	t-test	P-value
Urinary estradiol level	Baseline	336.88±183.034	327.6±234.73	-0.201-	0.841
	LD (35 mg)	84.1±58.47	114.28±90.93	0.352	0.727
	HD (70 mg)	38.87±27.293	56.57±36.11	-0.281-	0.780
P-value according to soymilk intake		**0.02**	**<0.0001**		
Urinary estrone level	Baseline	83.31±55.01	83.31±64.25	0.480	0.634
	LD (35 mg)	87.68±75.277	89.96±73.665	-0.085-	0.933
	HD (70 mg)	209.25±160.647	211.88±153.024	-0.159-	0.875
P-value according to soymilk intake		**<0.0001**	**<0.0001**		
Urinary genistein level	Baseline	740.64±451.47	1274.76±861.363	1.760	0.086
	LD (35 mg)	1885.21±1512.886	1711.79±1538.71	0.561	0.578
	HD (70 mg)	4443.03±3010.25	4077.95±3749.37	-0.519-	0.606
P-value according to soymilk intake		**<0.0001**	**<0.0001**		
Urinary daidzein level	Baseline	2305.35±2238.06	2760.61±2376.166	0.925	0.36
	LD (35 mg)	3690.66±2588.255	5529.65±4594.37	2.132	0.039
	HD (70 mg)	5253.4±3709.04	23181.07±13194.75	2.248	0.03
P-value according to soymilk intake		**0.002**	**<0.0001**		

Note: * – Four cases missed follow-up. Baseline (control), low dose (LD) 35 mg and high dose (HD) 70 mg of soy isoflavone as soymilk.

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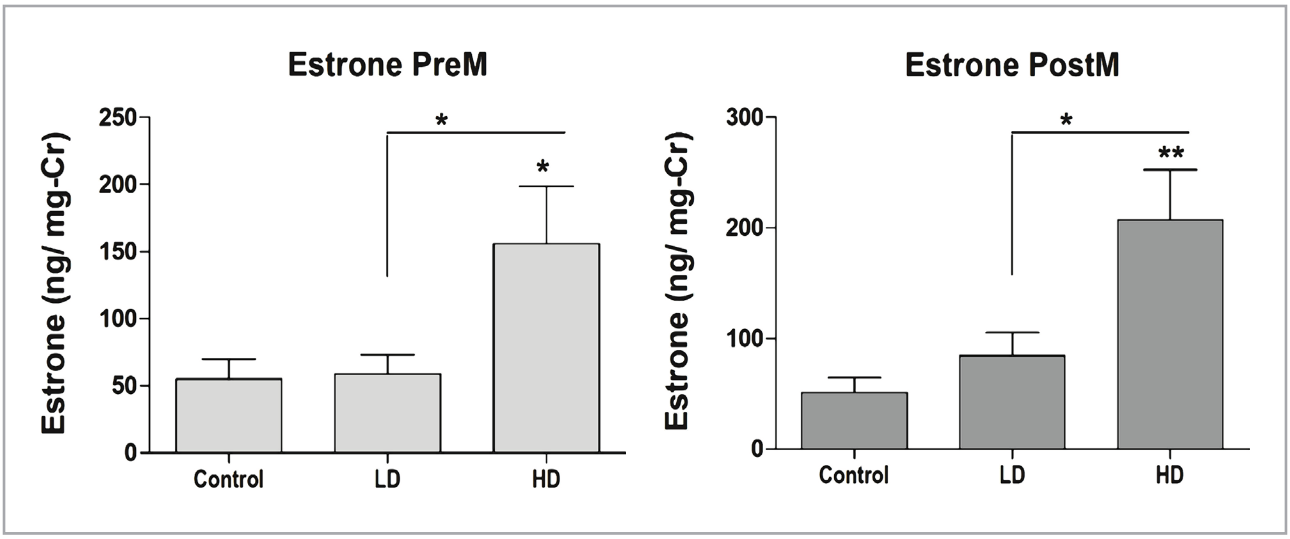

Figure 2: Urinary estrone level according to soy isoflavone intake in premenopausal and postmenopausal women. Control, low dose (35 mg) and high dose (70 mg) of soy isoflavone as soymilk. Data illustrated as mean±SEM. (*** – p<0.0001, ** – p<0.001 and * – p<0.05) linked to the control.

low dose (35 mg) and one-month consumption of high dose (70 mg) of soy isoflavones as soymilk.

Furthermore, there is no significant difference in urinary genistein levels between PreM and PostM women, at baseline, after one-month consumption of low dose (35 mg) and one-month consumption of high dose (70 mg) of soy isoflavones, as demonstrated in Table 3.

Urinary level of Diadzein

The results of this study revealed a significant (p=0.002 and p<0.0001) increase in urinary diadzein levels in PreM and PostM women, respectively, after one-month consumption of low dose (35 mg) and one-month consumption of high dose (70 mg) of soy isoflavones as soymilk.

Furthermore, urinary diadzein level is significantly (p=0.039 and p=0.03) higher in PostM than PreM after one-month consumption of low dose (35 mg) and high dose (70 mg) of soy isoflavones, respectively, as demonstrated in Table 3.

Urinary isoflavones correlation with urinary estradiol and estrone levels

This study's results demonstrated no significant correlations between urinary genistein and diadzein levels with urinary estradiol and estrone levels, as demonstrated in Table 4.

Table 4: Urinary isoflavones correlation with urinary estradiol and estrone levels in pre and postmenopausal women.

		Urinary genistein			Urinary daidzein		
		Baseline	LD	HD	Baseline	LD	HD
PreM	Urinary Estradiol	r=-0.22 p=0.26	r=-0.03 p=0.87	r=-0.36 p=0.07	r=-0.03 p=0.87	r=-0.07 p=0.72	r=-0.16 p=0.43
	Urinary Estrone	r=-0.21 p=0.28	r=-0.05 p=0.78	r=-0.06 p=0.75	r=0.18 p=0.36	r=0.04 p=0.83	r=-0.13 p=0.51
PostM	Urinary Estradiol	r=0.24 p=0.20	r=0.008 p=0.96	r=-0.007 p=0.97	r=-0.02 p=0.92	r=0.01 p=0.96	r=-0.11 p=0.56
	Urinary Estrone	r=0.19 p=0.33	r=0.26 p=0.18	r=-0.11 p=0.59	r=-0.28 p=0.14	r=-0.32 p=0.96	r=-0.13 p=0.49

Note: Premenopausal (PreM), postmenopausal (PostM) women, baseline (control), low dose (LD) 35 mg and high dose (HD) 70 mg of soy isoflavone as soymilk. Statistically significant values (significance P<0.05).

Discussion

A randomized, interventional comparison study was carried out on 120 Iraqi females aged 25 to 75 years with hormone-positive breast cancer. About 72 were PostM women with a mean age of (55.2±8.12) years and 48 were PreM women with a mean age of (37.91±3.476) years recruited to this study. Age is an important factor in the incidence and treatment of breast cancer [25]. In most Arabian regions, breast cancer is more commonly detected in women under the age of 50, unlike the Western countries, where women aged 50 years and older are most commonly diagnosed [26]. It has been proposed that these differences are due to changes in exposure to hormones, diet, physical activities, and other risk factors such as ethnicity, religion and localities [27]. Younger generations are continuously detected with breast cancer, which has been comprehensively shown in the Iraqi Cancer Registry [28].

The current study recognized that the BSA of PreM women is significantly (P=0.003) higher than PostM women and these results are in accordance with previous Iraqi studies [29–31].

Concerning marital status, about 106 (88.3%) women in this study were married; this was inconsistent with the previous study, where 69.4% of women with breast cancer were married [32]. Furthermore, there was no significant difference between PreM and PostM regarding employment.

In relation to comorbid conditions, there was a highly significant (P <0.0001) increase in PostM than in PreM women; the association of comorbid diseases with older age and hormonal changes could explain this. Furthermore, there was no significant difference between PreM and PostM regarding family history. Approximately 33.3% of women in this study had a positive family history of breast cancer, consistent with previous studies [32–35].

The strongest predictors of distant metastasis, disease-free survival, and overall survival of breast cancer are predictively influenced by tumor size (T stage) and lymph node status, which correlate strongly with time to progression and prognosis [36].

The overall mean duration of hormonal therapy of PostM women was statistically higher (P=0.004) than the overall mean duration of hormonal therapy of PreM women. A previous study confirmed anastrozole's long-term advantage and safety over Tamoxifen as adjuvant therapy for PostM women with hormone-positive breast cancer [37]. Aromatase inhibitors are an even more effective endocrine treatment than Tamoxifen for postmenopausal women, with further proportional reductions in recurrence rates of about 30% [38].

The results of this study revealed a significant (p<0.0001) much decline in urinary estradiol levels in PreM and PostM women after one-month consumption of low dose (35 mg) and one-month consumption of high dose (70 mg) of soy isoflavones as soymilk. In addition to a significant (p<0.001) much increase in urinary estrone level after one-month consumption of a high dose (70 mg) of soy isoflavones as soymilk. However, there is no significant difference in urinary estrone level after one-month consumption of a low dose (35 mg) of soymilk. Furthermore, there is no significant difference in urinary estradiol and estrone levels between PreM and PostM women, at baseline, after one-month consumption of low dose (35 mg) and one-month consumption of high dose (70 mg) of soy isoflavones as soymilk.

These findings are in agreement with a previous study that investigated the effect of soya isoflavone consumption in PreM and PostM women. They suggested that the mechanisms of bimodal alteration involve modulation of estrogen metabolism away from the production of potentially carcinogenic metabolites [16a-(OH) estrone, 4-(OH) estrone, and 4-(OH) estradiol] in PreM women, whereas, in PostM women, the mechanism includes raised the ratio of 2/4-(OH) estrogens and declined the ratio of genotoxic: total estrogens. As a result, soya constituents may exert cancer-preventive effects in PostM women by altering estrogen metabolism away from genotoxic metabolites toward inactive metabolites, which supports this study's findings [39]. These findings are also in agreement with another study that investigated the effect of soy isoflavones on estrogen metabolism, and they found that both genistein and daidzein are strong inhibitors of estrone and estradiol sulfation but delayed estradiol glucuronidation to a lower extent [40]. In addition, our findings align with previous studies linked between estrone, estradiol, genistein and daidzein and breast cancer risk and progression [41].

However, our findings disagree with a meta-analysis proposed that in PreM women, soy isoflavones intake did not affect estrone, estradiol or sex hormone binding globulin concentrations but decreased LH and FSH. While in PostM women, there is no significant effect of soy isoflavones intake on estrone, estradiol, sex hormone binding, LH and FSH [42].

To the best of our knowledge, this is the first time study to determine the association of urinary estrogen

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levels with urinary isoflavone levels in hormone-positive breast cancer PreM and PostM women treated with anti-estrogens, particularly in Iraq and generally in Eastern Mediterranean countries.

Conclusion

Estrone level has biphasic non-significant change after isoflavone intake. Estrodail and Didazain level in PreM women is lower than in PostM women after soy intake. Genstaine level in PreM women is higher than in PostM women beyond soy intake, as most of the eastern studies proved that patients who experience low urinary estradiol levels in soy food have less tendency to disease progression.

Acknowledgments

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Conflict of interest

The authors declare no conflict of interest.

Ethics approval

The approval for this study was obtained from the Ethics Committee of the College of Pharmacy, University of Basrah (approval ID: 3/5/293, October 2021).

Consent to participate

Written informed consent was obtained from the participants.

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