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The Comparative Study of the Administration of the Combination Preparation of Isoflavones and Hyaluronic Acid in Menopausal Women for the Treatment of the Symptoms of Menopause, Urogenital Atrophy and Oteoporosis in Relation to Existing Hormone Replacement Therapies

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ABSTRACT

Background: Menopause is characterized by a series of symptoms and effects from the various systems and organs, for which, the decline in estrogen production from the ovaries is considered responsible. **Objective:** The aim of this study was to make comparative study of the administration of the combination preparation of isoflavones and hyaluronic acid in menopausal women for the treatment of the symptoms of menopause, urogenital atrophy and osteoporosis in relation to existing hormone replacement therapies. **Methods:** In this five-year, double-blind, placebo-controlled clinical study, a total of 274 postmenopausal women were enrolled and classified into three groups. Participants in group A, were 96 women who did not receive Hormone Replacement Therapy (HRT), in the second group, 92 received daily treatment with tibolone (2.5 mg) as monotherapy, and in the third group, 86 received treatment with a pharmaceutical formulation of hyaluronic acid 120 mg and isoflavones. MF11RCE 80 mg. **Results:** In the postmenopausal women of our study, a significant reduction of postmenopausal symptoms was found in both groups B and C of participants who received hormone replacement preparations compared to group A who did not receive HRT. Furthermore, no difference in efficacy was observed between the administered preparations of isoflavones and tibolone. **Conclusion:** The combination of isoflavones and hyaluronic acid has the same efficacy as tibolone in menopausal symptoms.

Key words: treatment options, climacteric symptoms, isoflavones, hyaluronic acid, hormone replacement therapy.

1. BACKGROUND

Menopause should be treated as a state of differentiation of estrogen metabolism.

Chronologically, it is divided into three periods. First the premenopausal period is characterized by a decrease in progesterone and an increase in estrogen. Then menopause itself, which is characterized by a fall in estrogen, and finally the period after menopause that follows with the classic symptoms of menopause. This transitional phase that begins before menopause and continues after it, during which a woman goes beyond the reproductive stage, is properly called climacteric (1, 2).

Hyaluronic acid moisturizes/lubricates moving parts of the body, especially joints and muscles. It is also called "nature's moisturizer" because of its excellent moisturizing properties, especially when used in skin care products. It also acts as a "cushion" for nerves and bone endings, while it is a natural barrier for the spread of diseases (3-6).

MF11RCE isoflavones are widely used for the prevention and treatment of various disorders related mainly to women's health and especially osteoporosis and menopausal discomforts, based on epidemiological studies that brought the above diseases to a lower percentage, in populations with a high consumption of these plant estrogens (3-6).

MF11RCE isoflavones uses a plant-based, not animal-based, form of hyaluronic acid. The skin is the most important field of action of hyaluronic acid. 50% of its total is in the skin. It contributes to the proper lubrication of the lubricating fluid of the body's joints (articular fluid) and the vitreous gel of the eye. Hyaluronic acid and collagen are vital elements for the structure and layers of the skin. Collagen gives firmness to the skin and hyaluronic acid nourishes and hydrates the collagen (3-6). Hyaluronic acid works by filling the voids in the skin, as it binds with water and therefore fights wrinkles. However, the dosage that will be used is the one recommended by National Drug Agency (NDA), 1 tablet per day, (see NDA document). It also intends to determine the treatment time required for each patient as well as its position as a monotherapy or complementary treatment and compare it to current existing hormone replacement therapies in the treatment of menopausal symptoms if the dosage recommended by the NDA is not sufficient (7-12).

The purpose of this study is to investigate the activity of this medicinal preparation of hyaluronic acid 120 mg and isoflavones MF11RCE 80 mg, tablets, hyaluronic acid - MF11RCE, in its contribution to the recovery of the symptoms of menopause and the treatment of its secondary complications such as osteoporosis, urogenital atrophy, and accompanying urinary incontinence and vasomotor syndrome which are common conditions in these women (12-14).

2. OBJECTIVE

The aim of this study was to make comparative study of the administration of the combination preparation of isoflavones and hyaluronic acid in menopausal women for the treatment of the symptoms of menopause, urogenital atrophy and osteoporosis in relation to existing hormone replacement therapies.

3. MATERIAL AND METHODS

This case-control study was conducted at a tertiary university hospital of Democritus University in Greece. In the period from 01.02.2018 to 28.02.2023 approximately 274 postmenopausal women were included in this study, which were allocated into three groups.

In Group A (in which 96 postmenopausal women were included) was not administered HRT (hormone replacement therapy) preparation, while in group B (in which 92 postmenopausal women were included) and Group C (in which 86 postmenopausal women were included) were administrated Tibolone 2.5 mg and combination preparation hyaluronic acid 120 mg and isoflavones respectively. MF11RCE 80 mg one hyaluronic acid 120 mg and isoflavones. MF11RCE 80 mg.

Evaluation of the effect treatment therapy

A questionnaire with objective findings: dyspareunia, vaginal dryness, urinary incontinence, itching, burning, vaginal discharge, inflammation-infections, Sexual dysfunction questionnaire type FSFI (Female Sexual Function Index) and FSDS-R (Female Sexual Distress Scale-Revised) of ACOG, Cytological examination of vaginal smear and mapping of the cells according to their layers of origin. The evaluation parameters included: age, body, weight, parity, metabolic diseases, vitamin, ca, administration, surgical menopause (hysterectomy + adnexectomy) (s) versus (n), menopause, hot flashes, excessive sweating (night sweats), feeling of palpitations, dizziness, headache, nervousness, depression, insomnia, fatigue, musculoskeletal athralgias, changes in skin and hair, paraesthesia in oral cavity, anxiety, difficulty in sleeping, dryness of the vagina, loss of desire for sex, pain during intercourse, difficulty concentrating, weak memory, urological problems, symptoms of cystitis, urinary tract, infection, stress, incontinence, osteoporosis, bone density, thromboembolic diseases, findings from mammography.

The above parameters were in a questionnaire completed by the participating postmenopausal women at the beginning of the follow-up and at quarterly follow-ups for one year. Laboratory tests were done once after 6 months of follow-up and at the end of follow-up after one year.

Selection Group for MF11RCE isoflavones treatment - Exclusions from treatment

- His age is less than 24 years and more than 64 years.
- Women who received: a) Psychological treatments for dyspareunia and anorgasmia; b) Use of estrogen in the form of topical gels; and c) Use of testosterone.
- MF11RCE isoflavones treatment is contraindicated in:
- Pregnant women;
- In women with vaginal, cervical, pelvic infections and urinary tract infections;
- In women with surgeries of the genitourinary system during the previous six months or year;
- In women with malignant disease;
- To women who cannot understand the effectiveness of therapy (12-14).

Ethical approval for this prospective study was confirmed by the Ethics committee of the University Hospital in Alexandroupolis, Democritus University of Thrace, (Alexandroupolis, Greece; reference no. 951/15/10/2018). All patients provided written informed consent gave their written consent for their participation in the present study.

We performed a retrospective study to analyze the final outcome of menopausal women after the provision of medication. The data were analyzed with SPSS software. Relative (%) and absolute frequencies were presented for qualitative variables, while quantitative variables were presented using mean ± standard deviation. The Chi-square test was used for the univariate analysis of qualitative variables. Differences were considered as statistically significant at a p-value < 0.05. SPSS 23 for Windows was used for the analysis.

4. RESULTS

Finally, data from 274 menopausal women were used in our study. The mean age of the participants was 51,8 years old (min=46, max=58, SD±3,33). The mean weight of the women was 78,14kg (min=67, max=103, SD±7,05). 35% (n=96) did not take any medication, 33,6%(n=92) used livial, a medication containing tibolone, and the rest 31,4%(n=86) used menopearl, a medication containing Isoflavones and Hyaluronic acid. More than half of the participants (54,4%, n =149) had two childbirths, 36,9% (n=101) had one, 7,7% (n=21) and 1,1% (n=3) had three and four childbirths respectively (Table 1).

More than 2/3 of the women (71,9%, n=197) did not have a metabolic disease and almost half (50.7%, n=139) did not use any vitamin and calcium supplement. 48,9% (n=134) had a surgical cause of menopause, due to hysterectomy and adnexectomy (Table 2).

		N	%
Medication used	No medication	96	35,0
	Livial	92	33,6
	Menopearl	86	31,4
Number of childbirths	I	101	36,9
	II	149	54,4
	III	21	7,7
	IV	3	1,1
Age(years, mean, SD)		51,8 ± 3,33	
Weight (mg, mean, SD)		78,14 ± 7,05	

Table 1. Characteristics of the sample in our reserach study

	N	%
Metabolic diseases		
YES	77	28,1
NO	197	71,9
Vitamin and Calcium supplements		
YES	135	49,3
NO	139	50,7
Causes of menopause		
Normal procedure	140	51,1
Surgical procedure	134	48,9

Table 2. Medical condition of the responders

We tried to examine if the provision of medication for menopause has a positive impact on the symptoms and the medical follow-up of the participants. The women were asked if they were having hot flashes, excessive sweating (night sweats), feeling of palpitations, dizziness, headache, nervousness, depression, insomnia, fatigue easily, musculoskeletal symptoms, arthralgias, changes in skin and hair, or in the oral cavity. Furthermore, they were asked to mention if they are feeling paraesthesia, symptoms of anxiety, difficulty in sleeping, dryness of the vagina, loss of desire for sex, pain during intercourse, difficulty in concentration, weak memory or urological problems and symptoms of cystitis-Urinary tract infection or stress incontinence. We found that the provision of medication is positively correlated with improvement of menopause symptoms($p<0,001$). Similar results were found when we tried to examine the effect of medication provision on bone mineral density (BMD). We have to mention here that more than half of the participants not taking medication for menopause (52,1%, n=50) had abnormal BMD (Table 3).

None of the participants had thromboembolic diseases, abnormal mammography, or abnormal laboratory examinations (data not shown).

As a second analysis of the previous question, we tried to examine if there is a difference between the 2 different medications provided for menopause. No differences were found between the 2 medications; similar percentages of participants did not present symptoms (Table 4).

5. DISCUSSIION

The preparations administered to the HRT mainly contain steroid reproductive hormones, i.e. estrogen either alone (mainly in the 1950s-1960s) or in combination with progestogens or androgens. The same category includes tibolone and phytoestrogens, while raloxifene is a non-steroidal substance (15, 16).

Tibolone is a synthetic steroid derivative of 19 nor testosterone that exhibits simultaneous estrogenic, progesteronic and androgenic effects. The degree of affinity for estrogens is small for tibolone as a whole and for the 3a and 3b hydroxy isomers, for progesterone and androgens it is small for tibolone as a whole, moderate for the D4 isomer while there is no chemical affinity for the 3a and 3b hydroxy isomers (17, 18).

The advantages of the method include the reduction of climacteric vasomotor symptoms and estrogenic effect on bone tissue, the compensation of estrogenic action in the endometrium and improvement of the desire for sexual intercourse. Its disadvantages include the fact that the degree of its effect on other tissues and especially on the breast is not yet known. Tibolone is administered in a daily dose of 2.5 mg for the relief of symptoms and the control of uterine bleeding (17-22).

Raloxifene and bazedoxifene are classified as SERMS (selective estrogen receptor modulators). Their action is both agonistic and antagonistic to estrogen depending on the targeted tissue. In particular, it mimics the action

Variable	Provision of medication for menopause		
	Yes (%)	No (%)	P-Value
Hot flashes, Excessive sweating (night sweats), Feeling of palpitations, Dizziness		`	
Yes	28 (15,7)	82 (85,4)	<0,001
No	150 (84,3)	14 (14,6)	
Headache, Nervousness, Depression, Insomnia			
Yes	0 (0)	82 (85,4)	<0,001
No	178 (100)	14 (14,6)	
Fatigue easily, Musculoskeletal symptoms, Arthralgias Changes in skin and hair or oral cavity			
Yes	0 (0)	89 (92,7)	<0,001
No	178 (100)	7 (7,3)	
Paraesthesia, Anxiety, Difficulty sleeping			
Yes	0 (0)	89 (92,7)	<0.001
No	178 (100)	7 (7,3)	
Dryness of the vagina, Loss of desire for sex, Pain during intercourse, Difficulty concentrating, Weak memory			
Yes	0 (0)	89 (92,7)	<0.001
No	178 (100)	7 (7,3)	
Urological problems Symptoms of cystitis Urinary tract infection stress incontinence			
Yes	0 (0)	89 (92,7)	<0.001
No	178 (100)	7 (7,3)	
Bone Mineral Density			
Normal	177 (99,4)	46 (47,9)	<0.001
Abnormal	1 (0,6)	50 (52,1)	

Table 3. Univariate analysis

of estrogen on bone tissue, the cardiovascular system and lipid metabolism. This action is different from that of 17β estradiol and tamoxifen (23, 24).

They show an anti-estrogenic effect on the endometrium and have an inhibitory binding effect on the estrogen receptors, antagonizing the estrogen-dependent proliferation of MCF-7 cells of breast tumors. Their advantages include the absence of mitotic activity in the breast and endometrium. Other advantages are the fact that they can be administered without progestogen to women with an intact uterus and act adequately in the prevention of osteoporosis. Finally, they do not affect ovarian estrogen production and the hypothalamic-pituitary axis (23, 24).

Their disadvantages include the unsatisfactory treatment of climacteric symptoms and the occurrence of adverse effects such as hot flashes and leg cramps. Based on the above, they are the treatment of choice for the prevention of osteoporosis in cases of high risk for breast cancer and in cases of bleeding or spotting during conventional treatment (23, 24).

Phytoestrogens are plant components with estrogenic activity and potential positive therapeutic intervention in menopausal symptoms. The most well-known source of origin is vegetables and especially soy. According to epidemiological studies, they have a favorable effect on the remission of vasomotor symptoms. On the contrary, they are less effective in terms of their action in the atrophy of the genitourinary system. It has been reported that in women with mild and moderate symptoms and a contraindication to the application of HRT, the enrichment of the diet with foods rich in phytoestrogens may be beneficial. Finally, in the long term, soy may protect the cardiovascular system while, on the contrary, its effect on osteoporosis needs further investigation (9, 25-28).

Isoflavones are derived from plants and structurally or functionally resemble endogenous-natural estrogens and their active metabolites. They also have significant estrogenic (agonistic/antagonistic) activity.

The main groups of phytoestrogens are isoflavones (genistein, daidzein, equol, biochanin A), lignans (enterolactone, enterodiol), coumestans (coumestrol), stilbones (resveratrol) and flavonoids (quercetin, kaempferol). They are polyphenols, there are >8000 phenolic structures in the plant kingdom and they occur as simple phenolic rings or as polymers (tannins). Flavonoids are divided into 6 subclasses and except for some they do not have estrogenic properties. Their important sources are grapes, apples, citrus fruits, onions, berries, broccoli, soybeans, tea, coffee, cocoa and red wine. Stilbones are used in non-steroidal synthetic estrogens such as Diethylstilbestrol (DES) (29-34).

The most studied isoflavones Genistein and Daidzein come from soy foods that are mainly consumed in the East, while lignans (linseed, sesame, rye and cereals in general, legumes, and vegetables) are a large part of the diet of Europeans. Their quantity in nature depends on

Variable	Medication used		
	Livial (%)	hyaluronic acid - MF11RCE **(%)**	P-Value
Hot flashes, Excessive sweating (night sweats), Feeling of palpitations, Dizziness		`	
Yes	15 (16,3)	13 (15,1)	0.828
No	77 (83,7)	73 (84,9)	
Headache, Nervousness, Depression, Insomnia			
Yes	0 (0)	0 (0)	-
No	92 (100)	86 (100)	
Fatigue easily, Musculoskeletal symptoms, Arthralgias Changes in skin and hair or oral cavity			
Yes	0 (0)	0(0)	-
No	92 (100)	86 (100)	
Paraesthesia, Anxiety, Difficulty sleeping			
Yes	0 (0)	0 (0)	-
No	92 (100)	86 (100)	
Dryness of the vagina, Loss of desire for sex, Pain during intercourse, Difficulty concentrating, Weak memory			
Yes	0 (0)	0 (0)	-
No	92 (100)	86 (100)	
Urological problems Symptoms of cystitis Urinary tract infection stress incontinence			
Yes	0 (0)	0 (0)	-
No	92 (100)	86 (100)	
Bone Mineral Density			
Normal	91 (98,9)	86 (100)	0.332
Abnormal	1 (1,1)	0 (0)	

Table 4. Comparison of Livial and hyaluronic acid - MF11RCE. Variable Medication used

the genetics of the plant and environmental factors. Of course, in Europe, there is a large consumption of soy-based foods, and according to the literature in the various countries, the intake ranges from 20-100mg per day and is under research for their use as natural selective modifiers of ERα, ERβ (29-34).

Individual differences in the bioavailability of the isoflavones genistein, and daidzein, further to equol (in 30%-50% of people), or to o-demethylangolensin (O-DMA) in 80%-90% of the population. The metabolite of genistein is p-ethyl phenol. The main phytoestrogens detected in the blood and urine of mammals are daidzein, genistein, equol, O-DMA. Their metabolism, mainly intestinal, and hepatic differs between children, adolescents and adults, resulting in difficulty in the interpretation of the various measurements (29-34).

Their role is not completely known even for plants (protection from UV radiation and fungi, antioxidant activity, and many others). Associated with ERs (α, ERβ), they have a selective modifying capacity in the final gene expression, acting on transcription factors, so that their estrogenic/anti-estrogenic effect is tissue-specific and cell-specific. They are referred to as natural SERMs. They are weak estrogens, but the affinity and activation of ERβ is 100 times stronger than that of ERα. They are therefore considered to have a beneficial effect on tissues with a strong presence of ERβ (ovary, prostate, lung, CNS, bladder, gastrointestinal). In cancerous tumors, the existence of ERβ is favorable for the course of the disease. They also inhibit enzymes important for steroid metabolism, such as β-HSDs, (12 isoenzymes, 1,5,7 are of interest to the breast), 3β-HSD1, aromatase, sulfatase, and sulfotransferases, which convert weak estrogens and androgens, in strong estrogens with mitogenic action (29-34).

Like estrogens, they bind to membrane receptors, but they also exert estrogen-independent action by other mechanisms. Genistein may alter the expression of progesterone, androgen, and oxytocin receptors with unknown clinical significance. It has been reported that they induce the release of hormones from SHBG, inhibit MAPkinase, topoisomerase II etc. They exert unwanted effects on the reproductive system, female and male. Also, chronic exposure of spermatozoa to high doses of genistein caused infertility, by inhibiting the acrosomal reaction and affecting their motility.

In in vitro tests they stimulate osteoblasts and suppress osteoclasts through IL-6, OPG, RANKL. For the study of its action, the synthetic isoflavone, did not affect BMD and vertebral fractures, in 475 menopausal women, at a dose of 600mg for 4 years. Other authors observed with the same formulation, an improvement in osteoporosis indicators Phytoestrogens may prevent peri-menopausal bone loss, but there is no clinical evidence for the prevention of osteopenia, and reduction for the risk of fractures (35-38).

The treatment of hot flashes in women with breast

cancer is a particularly difficult clinical problem, which will increase over time, for two main reasons: a) the increase in survival, and b) the increase in the number of very young affected women, of whom the vasomotor effects are more intense, with consequent impact on the quality of life. In in vitro, preclinical and clinical studies the results are highly contradictory. The purity of the phytoestrogen form increases the stimulatory estrogenic effect and reverses the effect of tamoxifen (35-38).

A recent study showed a strong inhibitory effect on 17β-HSD1, which converts E1 to E2, of 10 flavonoids, as well as intermediate products of their biosynthesis, while none had a proliferative effect on breast cancer cells. They seem to protect only women who are exposed from an early age, from fetal possibly, in reproductive life studies come to conflicting conclusions, while for menopause there are also insufficient results for their potentially harmful effect (35-38).

In in vitro and in vivo studies there is a neuroprotective effect through selective activation of ERβ. A study of young women who received for 1 week a diet rich in phytoestrogens with a intake of 160mg/day showed no effect on various intellectual skills, while for 12 weeks it significantly improved hot flashes, mental mood, physical condition, but did not affect lipids and mental functions at all (35-38).

In January 2009 the Cochrane Database of Systematic Reviews published results of 30 studies in peri- and postmenopausal women who received high doses of soy, soy extract, red clover extract (Promensil), and other phytoestrogens for at least 12 weeks. Of the 30 studies, very few met the criteria for inclusion in a meta-analysis. In these, no significant reduction of vasomotor symptoms was found compared to placebo, which, however, in several studies, had a marked improvement in both hot flashes and night sweats. In the remaining, low-quality studies, they observed a small reduction in discomfort always compared to placebo (39-42).

A Mayo Clinic pilot study was considered encouraging, in which 30 women received 40g of ground flaxseed daily for 6 weeks, and recorded a significant reduction (>50%) in their hot flashes score. However, a longer study in terms of duration and number of women is needed for definitive conclusions. In the above study, 50% experienced mild abdominal distention, 30% mild diarrhea, and 20% discontinued due to side effects. The authors do not consider that there is sufficient evidence to recommend phytoestrogens in the treatment of menopausal symptoms. Hyperplasia of the endometrium, for administration up to 2 years was not observed, but intake of soy 150mg daily, for 5 years caused hyperplasia (39-42).

According to research, the intake of hyaluronic acid reduces the levels of pyridolin (an indicator of bone density) in the urine, resulting in increased bone density. Before menopause, estrogen increases hyaluronic acid levels, maintaining healthy hair, skin and nails. During menopause it has been proven that the natural regulation of hormones with the parallel use of hyaluronic acid supplements contributes to the maintenance of natural beauty (40-42).

Glucosamine combines with a glucuronic acid molecule to form hyaluronic acid. Often the body cannot unite these two molecules and the production of the precious hyaluronic acid is limited. Hyaluronic acid supplementation maximizes the availability of the composition, enhancing the body's ability to rebuild (40-44).

Along with its amazing moisturizing properties, hyaluronic acid fills the gaps between the skin cells and produces a lasting toning, leaving the skin soft, smooth and elastic especially in the areas of the face and lips. In cases of premature aging, such as Hutchinson-Gilford progeria syndrome, an increase in hyaluronic acid secretion is observed 17 times more than in normal conditions (40-44).

Hyaluronic acid regulates the wound healing process and provides the necessary structural nutrition for the formation of new tissue. It is an element of vital importance for the reconstruction of skin tissue after its injury. Chondroitin sulfate increases the production of hyaluronic acid in the body, while in large quantities it achieves the successful reconstruction and treatment of heart tissue (42-46).

Part of the therapeutic mechanism of hyaluronic acid is the strengthening of the natural synovial fluid, making it slimier, its contribution to cartilage biosynthesis, its anti-inflammatory property and its direct analgesic effect. The body produces and eliminates several thousand mg of hyaluronic acid per day. With age and disease the rate of hyaluronic acid elimination does not keep pace with the rate of production, resulting in aging.120mg of hyaluronic acid is taken 1-3 times a day alongside meals to preserve youth and restore menopausal problems. Higher doses are not necessarily more effective. Taking 3-10mg for each kilogram of body weight is the recommended dosage with the most visible and immediate results (42-46).

Potential Application/Indications: Anti-aging, wrinkle prevention and control, wound healing, acne, post-surgical tissue repair, menopausal hair/nail health, nerve crush syndrome, osteoarthritis, osteoporosis, connective tissue dysfunction, sports and injuries, eye problems (especially presbyopia), possible genetic predisposition to premature aging (40-44).

Contraindications: Its use is not recommended for people suffering from hypertension or lymphedema due to cancer (40-44).

It has been reported that rheumatoid arthritis sufferers should avoid using hyaluronic acid supplements. The classic morning stiffness symptoms of the disease are due to an autoimmune reaction of shedding hyaluronic acid during sleep.Interactions: The simultaneous intake of diuretic drugs is not recommended, due to the hydrophilic nature of hyaluronic acid.Side effects: Drinking plenty of fluids at regular intervals is essential for those taking hyaluronic acid supplements (40-44).

The 1st prescription is usually three months followed by a 6-month administration, remission of vasomotor symptoms is observed after a few weeks of satisfactory treatment. The rate of improvement of urogenital symptoms is more slow. because approximately 6 months are

required for the estrogenization of the vagina. Some argue that it should be applied until the symptoms subside. Usually, a treatment of 3-5 years is sufficient for the complete remission of the symptoms without excluding the relapse upon interruption, in which case a repetition of the treatment is necessary. Relapse is inevitable with regard to the symptoms of urogenital atrophy Discontinuation of treatment without medical consent is often due to events that occur during this period at the woman's age, such as retirement or the appearance of breast cancer in a relative or friend (40-44).

However, in any case, for the continuation of the treatment for a long period of time, the possibly slightly increased risk of breast cancer and thromboembolic events should be taken into account.

It is difficult to conclude precisely according to the data so far whether there is a relationship between HRT and breast cancer. However, it is reasonable to assume that the relative risk increases by 1-2% per year of use. Therefore, the decision to apply HRT or not should take into account the above risk in relation to the benefits that may arise.

However, it is reasonable to assume that the relative risk increases by 1-2% per year of use. Plus. Many consider it an absolute contraindication to the application of HRT in high-risk women, especially when there is a burdensome family history (40-44).

Recently, research has expanded towards the administration of SERMs with the main objective of reducing the incidence of breast cancer due to the anti-estrogenic effect of the above substances in the mass gland with quite promising results.HRT is associated with an increased risk of venous thrombosis that depends on individual and treatment-related factors. Transdermal administration of estrogen appears to be associated with a lower risk compared to its oral counterpart. There is a questionable relationship between HRT and cardiovascular disease with the timing and duration of treatment influencing the course of the disease (40-42).

In general, the administration of HRT can reduce the risk of cardiovascular disease and in particular the risk of coronary heart disease by 50% if it is administered within 10 years of the onset of menopause. In general, estrogens have a favorable effect on the lipid profile by increasing HDL levels and reducing LDL levels. The action of progestogens is neutral or antagonistic to that of estrogen (42-46).

According to the finding of the National Institute for Health and Care Excellence (NICE) THO does not increase the risk and mortality from cardiovascular disease as long as it is given to women under the age of 60. The modern view regarding hormone replacement therapy is that administered to perimenopausal and early menopausal women without contraindications and after systematic information about the possibilities risks and benefits.

It is not recommended to start treatment after the age of 60. In women with early menopause <40 years old or early menopause <45 years old it is recommended the administration of replacement with sex hormones until the age of 51 for the treatment of vasomotor symptoms, and for the prevention of osteoporosis and cardiovascular diseases (42-46).

According to our results after 12 months, a significant improvement of menopausal symptoms was observed between the PLACEBO groups A and the other two groups B and C receiving hormone replacement preparations Livial and Isoflavones respectively ($P < 0.05$) (Table 3).

In all groups, the demographic characteristics, metabolic diseases, the percentages of receiving additional trace elements and vitamins as well as the percentages of participating women with natural or surgical menopause were similar, while the difference between the two groups was not statistically significant (Table 1 and 2). Supplemental non-hormonal trace element and vitamin preparations were not consistently taken continuously while hormone replacement and isoflavones were taken daily without interruption.

Participants in the women's study were followed for 12 months and no serious, moderate to severe adverse events were recorded. Serious adverse events were defined as those that resulted in death, life-threatening or permanent disability, and any adverse event that required hospitalization or intervention to prevent permanent damage.

Moderate to severe adverse events were defined as those in which symptoms were frequent (more than three times per day), lasted more than 1 hour, required prescription medication or frequent (more than three times per week) non-prescription medication, resulted in reduced normal activities and incapacitation, and/or resulted in hospitalization.

In a percentage of approximately 34.5%, isoflavones continue to be taken beyond the study. No difference was observed between groups B, C regarding the improvement of symptoms in relation to the start of treatment, ($P < 0.05$) (Table 3). It is noteworthy that there were no differences in the effect of the two compared hormone replacement preparations on the symptoms. Isoflavones with hyaluronic acid have the same favorable effect as the administration of HRT (Table 4).

Limitations of the study

The present study was conducted to determine the safety and efficacy of the combined administration of isoflavones with hyaluronic acid and HRT in postmenopausal women. The present study, however, has its limitations. The study was a year old and the long-term effects of isoflavones are yet to be determined.

6. CONCLUSION

To recap, according to our existing results, the combination of isoflavones and hyaluronic acid is well tolerated, has an overall safety profile comparable to that of HRT, and is a safe alternative.

Further multicenter prospective studies are needed to validate our results in larger sets of menopausal women.

- **Author's contribution:** D. P, gave substantial contribution to the conAception or design of the work and in the acquisition, analysis and interpretation of data for the work. H-P-J, D.P., R.J., J.P., D.Z., R.S., S.U., J.S.,, had role in drafting the work and revising it critically

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