Universitatea
Transilvania
din Brașov

INTERDISCIPLINARY DOCTORAL SCHOOL

Faculty of Medicine

Andreea BĂLAN (CIUBOTARU)

COMPARATIVE STUDY OF THERAPEUTIC ALTERNATIVES FOR THE SYMPTOMATOLOGY AND PATHOLOGY ASSOCIATED WITH MENOPAUSE

SUMMARY

Scientific supervisor

Prof. Dr. Lorena DIMA

BRAȘOV, 2024

TABLE OF CONTENTS

ABBREVIATIONS LIST

5-HT	5 hydroxytryptamine
AMPC	Cyclic AMP
AST	Aspartate aminotransferase
BMI	Body mass index
CRP	C-reactive protein
E2	Estradiol
ER	Estrogen receptor
ESR	Erythrocite sedimentation rate
FDA	Food and drug administration
FSH	Follicle stimulating hormone
HDL	High density lipoprotein
IU	International Units
JNK	Jun N-terminal kinase
LDL	Low density lipoprotein
LH	Luteinizing hormone
LP(A)	Lipoprotein a
MENQOL	Menopausal Quality of Life Score
MER	Membrane estrogen receptors
PR	Progesterone receptor
PRA	Progesterone receptor type A
PRB	Progesterone receptor type B
RANK	Type II membrane protein
RANKL	Type II membrane protein ligand
SERM	Selective estrogen receptor modulator
DBP	Dyastolic blood pressure
SBP	Systolic blood pressure
TG	Triglyceride
TGO	Aspartat aminotransferase
TGP	Alanin aminotransferase

BRIEF SUMMARY

Introduction: Menopause represents a physiological event in a woman's life that is defined by the irreversible cessation of menstruation, as a result of the decrease and, finally, the loss of cyclic ovarian activity. Multiple studies have been performed during the last years to analyze the utility of non-hormonal therapeutic alternatives in treating postmenopausal symptoms and pathologies. This paper is a synthesis of the effects of natural compounds on the menopausal symptoms.

Objective: The main objective of this thesis was to identify an optimal therapeutic scheme to improve the symptoms and the quality of life of menopausal women, based on natural, non-hormonal dietary supplements. Also, this study aimed to analyze the effects of dietary supplements in relieving the symptoms induced by menopause.

Material and methods: The research was carried out at the Clinical Hospital of Obstetrics and Gynecology "Dr. Ioan Aurel Sbârcea" from Brașov and it was designed as a prospective observational study. The subjects were followed up for a period of 8 weeks (during the period 2020-2022). The women were included into three groups: the first group consumed a dietary supplement supplement based on fermented soy extracts, the second group consumed the same soy supplements associated with liophilized royal jelly capsules, to relieve the menopausal symptoms, and the control group refused the administration of any dietary supplements. As this is an observational study, the choice of therapy was entirely up to the individual physicians and patients.

Results: The general data of the patients and the values of the analyzed variables were similar in all the study groups at the first visit, without statistically significant differences. Analyzing the patients in the control group, those who did not follow any kind of treatment, we noticed that all the variables remained approximately constant at both study visits. In the group that received only dietary supplements based on fermented soy extracts, after 8 weeks of treatment, a statistically significant improvement of these variables was observed, except for transaminase values, inflammatory markers, diastolic blood pressure and urine culture. In the third group, which received an association of dietary supplements based on fermented soy extracts and liophilized royal jelly capsules, the values of the transaminases and inflammatory markers remained constant after the treatment. The effect of the association between the two types of dietary supplements proved to be superior, the patients in this group presenting after 8 weeks of treatment significantly improved values of the total MENQOL score, as well as of its subdomains, except for the sexual domain. The number of daily hot flashes was lower in this group compared to the S group. HDL-cholesterol, LDL-cholesterol, total cholesterol and salivary cortisol values were lower than in the S group. The total DASS-21R score as well as the DASS-21R-Anxiety, DASS-21R-Depression, DASS-21R-Stress scores were improved in groups S and SLM after the treatment, but no statistically significant differences were recorded in comparison with the scores of the control group.

Conclusion: The use of natural food supplements has proven its efficiency in treating specific symptoms of the menopausal period, as well as in the normal functioning of carbohydrate and lipid

metabolism after entering menopause. Also, the low price and the low number of side effects make them excellent tools for the management of the symptoms in menopausal women.

CHAPTER I. INTRODUCTION

Menopause is a physiological event in a woman's life that is defined by the irreversible cessation of menstruation, because of the decrease and, finally, the loss of cyclic ovarian activity. The criterion used to define this biological stage of women is the absence of periods for at least 12 months (Johnson et al. 2019, Zhou et al. 2020). Until the actual menopause period, the body goes through a transition period of variable duration, with an average of about 4 years, characterized by irregular periods or amenorrhea. This transition to menopause is characterized by a series of specific changes, clinical, biological and of course, endocrinological (Bondarev et al. 2019). According to literature data, the age at which women enter menopause has a high degree of variability, this being influenced by a series of environmental, genetic, lifestyle factors, etc. and it was set between 45.5 and 47.5 years (Zhou et al. 2020).

Until now, multiple studies have been carried out on the topic of therapeutic alternatives useful in the treatment of menopause symptoms and pathologies specific to this period. The main objective of this doctoral thesis is the identification of an optimal therapeutic scheme for the relief of symptoms and the improvement of the quality of life of menopausal women, as well as the comparison of the positive and negative effects of different therapeutic agents.

CHAPTER II. MENOPAUSE- CHANGES IN THE HORMONAL PROFILE, CLINICAL ASPECTS AND ASSOCIATED PATHOLOGY

II. 1. Hormonal profile changes in menopause

Both during the actual menopause period and during the pre- and post-menopause periods, the endocrinological panel of the woman acquires novel nuances, so that all these periods are characterized by remarkable hormonal changes. In menopausal women, the concentration of E2 is significantly lower compared to the concentration of E2 in normally menstruating women. Also, in this category of women, the level of FSH registers impressive values, despite the fact that it should be suppressed by E2 (Burger et al. 2002).

The androgenic hormones have been studied for their effects on natural menopause, but their role still needs further studies in order to be fully described. Testosterone, androstenedione and SHBG (sex hormone-binding globulin) levels showed small, but not insignificant decreases during the transitional years (Weinberg et al. 2006). Regarding the progesterone, its serum level was studied in postmenopause and the researchers' conclusion identified a decrease in progesterone level during this period, in parallel with the E2 level and with the significant increase of FSH and LH.

II.2. Clinical aspects of menopause

The menopausal transition and the postmenopausal period are marked by a wide variability of symptomatology and are characterized by physical and psychological changes that, in certain cases, depending on the degree of each one, can become debilitating and can negatively influence the personal, social and sexual life of the women from this category.

II.2.1. Vasomotor symptoms

Vasomotor symptoms, represented by hot flashes and profuse night sweats are the most common manifestations of the peri- and post-menopausal period and have a variable duration, between 5 and 15 years after the cessation of the ovarian activity (Harlow et al. 2012). Hot flashes are characterized by marked vasodilation and night sweats of the head, neck, and upper chest. It is frequently associated with changes in the underlying heart rate, with tachycardia and electrocardiographic changes, and while the skin temperature increases by several degrees Celsius, the basal temperature is slightly decreased. The duration of these episodes can vary from 5 minutes to several hours and can be unique or repeated several times a day (Bruce et al. 2009).

II.2.2. Genitourinary syndrome

While vasomotor symptoms appear more frequently during the transition period and at the onset of menopause, genitourinary symptoms are more prominent after a few years after the onset of menopause and their severity increases with age (Harlow et al. 2012). Reduced vascularity, muscle relaxation and epithelial thinning of the genitourinary tract are the most important changes induced by the dramatic drop of E2 in menopause (Bruce et al. 2009). Also, the loss of elasticity of the tissues at the level of the vagina leads to a wide range of dysfunctions, the most suggestive of which are dyspareunia with variable severity and traumatic vaginal bleeding, occurring even after minor traumas (ex: sexual intercourse) explained by a deficiency of epithelial tissue. Vaginal atrophy and poor lubrication are two other types of signs that appear after menopause. They generate irritation, pain and varying degrees of discomfort and the changes in the vaginal pH, which becomes more alkaline during this biological period of the woman, favoring the genital and urinary infections (Panay et al. 2015).

II.2.3. Sexuality during menopause

Many women complain of a decreased libido during the menopausal transition or in the postmenopausal period, a fact that can negatively influence their couple relationships. Also, with the decrease in libido, a decrease in sensitivity can also occur in this category of women. The most common cause that generates this symptomatology is represented by hormonal deficiency, as the decrease in the concentration of estrogen and progesterone directly affects the uro-genital tract by inducing dyspareunia and the other aforementioned changes (Bruce et al. 2009).

II.2.4. Other physical symptoms in menopause

In addition to the already mentioned symptoms, this biological period of the woman is also marked by other troublesome symptoms that can negatively influence the quality of life. Headaches and migraines frequently occur during this period and are a common cause of referral to the doctor. Another common symptom during the menopausal transition is sleep disturbances. Many women complain of difficulty in falling asleep and frequent awakenings during the night, also waking up very early in the morning. Postmenopausal women's sleep disorders include insomnia, sleep apnea, and restless legs syndrome (Jehan et al. 2015).

II.2.5. Mental disorders during menopause

Psychiatric disorders occur with increased frequency during the menopausal transition and include depressive disorders, irritability, anxiety, decreased ability to concentrate, and partial or complete memory loss. Receptors for estrogen, progesterone and testosterone have been described at the level of some nerve centers and estrogen has been proven to have an effect on some neurotransmitters such as serotonin, glutamate and gamma-amino-butyric acid (GABA), which is why the changes in the serum level of it can influence and induce these mental symptoms (Bruce et al. 2009).

II. 3. Pathology associated with menopause

II.3.1. Osteoporosis

In postmenopausal women, osteoporosis and increased risk of fractures are frequent phenomena that occur due to rapid bone turnover and the combination of increased bone resorption and slowed bone formation. The essential cause of bone reabsorption is represented by the drastic drop in serum estrogen that crowns this biological stage in a woman's life. Low progesterone also contributes to reduced bone formation (Prior et al. 2011).

II.3.2. Breast cancer and endometrial cancer

Menopausal transition is a period marked by an increased risk for the occurrence of breast cancer and endometrial cancer in women in this category. The physiopathological mechanism by which the breast neoplasm develops is represented by the increased level of endogenous estrogen, following the administration of the hormone substitution therapy, balanced by progesterone (Prior et al. 2011). Endometrial cancer is a pathology that often begins in menopause, a neoplasia with increased sensitivity to hormonal influence, in the occurrence of which a critical role is played by the increased concentration of estrogen, unbalanced by progesterone.

II.3.3. Cardiovascular diseases and metabolic syndrome

Currently, any woman in the menopausal transition period has an increased risk of cardiovascular diseases and metabolic syndrome, but the pathophysiological mechanisms still require studies to be fully explained. Metabolic syndrome is defined as a group of disorders that includes hypertension, insulin resistance, glucose intolerance, dyslipidemia and obesity of varying degrees and represents a challenge in the medical world of industrialized countries. (Ford et al. 2002). Obesity, which is frequently developed with the cessation of ovarian activity, is the main causal factor in the development of metabolic syndrome, but the mechanism by which they are in a causal relationship is not fully elucidated (Spiegelman et al. 2001, Khan et al. 2011).

CHAPTER III. THE PHARMACOLOGY OF ESTROGEN AND PROGESTERONE RECEPTORS

III.1. Estrogen receptors

During the last decade, the understanding of the mechanism of action of estrogen receptors has increased, both from a clinical and a paraclinical point of view. The signaling mechanism of estrogen is based on the interaction and interdependence of the two types of estrogen-specific receptors, ERα and ERβ (Heldring et al. 2007). Estrogen plays an essential role in the development and maintenance of normal sexual activity and also exerts a wide range of biological activities within the cardiovascular, musculoskeletal, immune and central nervous system (Gustafsson 2003). The most potent type of estrogen produced in the body is 17β-estradiol (E2), whose metabolites, estrone and estriol, are weak estrogen receptor (ER) agonists despite of their high affinity for these receptors (Heldring et al. 2007).

ER α was discovered in 1950 by Elwood Jensen (Jensen 1962) and for a long time it was believed that this receptor was the only mediator of estrogen signaling. But, about 30 years after its discovery, it was demonstrated that life is possible in its absence and that there is still another type of estrogen receptor (ERβ), capable of replacing the lack of the first discovered and described (Kuiper et al. 1996). Estrogen receptors can have antagonistic actions, and this finding suggests that the body's overall response to E2 is the result of a balance between ERα and Erβ signaling (Liu et al. 2002).

III.1.1. Types of estrogen receptors

The ERα receptor contains 595 amino acids in its chain and has two isoforms, hERα-46 and hERα-36, respectively, which lack the NH2-terminal domain (Wang et al. 2005). The percentage of similar sequences between wild-type hERα and hERβ is indicated in hERβ. The hERβ isoform contains 530 amino acids in the chain and is considered a full-chain wild-type isoform (Leygue et al. 1998). The rat and mouse ERβ isoforms consist of 549 amino acids and have 99% sequence similarity. The mERβ2 and rERβ2 isoforms contain an extra 18 amino acids at the level of the ligand binding site, causing a significant decrease in its affinity (Lu et al. 2000).

III.1.2. Physiology of estrogen receptors and their role in breast cancer

Another effect of estrogen is its involvement in promoting the growth and survival of breast tumor cells, which contain ERα, ERβ, and progesterone receptors. First, estradiol inhibits UV- or paclitaxel-induced apoptosis of breast cancer cells. Apoptosis involves an increase in JNK (Jun N-terminal kinase) which in turn inactivates the antiapoptotic proteins Bcl-2 and Bcl-xl. This leads to the activation of caspases and ultimately to cell death. The ability to inhibit JNK is one of the differences between the two estrogen receptors, so while the ERα isoform inhibits JNK, the ERβ isoform does not (Coriano et al. 2018). The role of membrane receptors in tumor cell survival is controversial. For example, in breast cancer, estrogens cause an increase in cAMP levels, but the consequences of this action vary depending on the level of ERα expression. In the cytoplasm of cells with a low expression of ERα, low concentrations of cAMP have been described regardless of the concentration of E2 in the culture medium. In subpopulations with higher ERα expression, low concentrations of E2 stimulated cell division, while higher concentrations of E2 inhibited it (Soltysik et al. 2013).

III.1.3. Pharmacology of estrogen receptors

Estrogen has a selective action depending on the target tissue, an aspect of paramount importance for the development of optimal therapies for the prevention and treatment of breast cancer, for postmenopausal hormone replacement and for optimizing fertility. Certain compounds that act through estrogen receptors, now called selective estrogen receptor modulators (SERMs), can demonstrate major differences in estrogen activity on different target tissues, in that these compounds can act as agonists on certain tissues and as antagonists on others. The development of optimal SERMs should take into account the two subtypes of estrogen receptors, ERα and ERβ that have different affinities and different responsiveness to different SERMs, as well as different tissue distribution (Carley et al. 2003). Since ERα and ERβ can form heterodimers when both are present in the same cell, the profile of recruited co-regulatory proteins may be different in a hormone-dimer complex versus a hormone-receptor complex (Katzenellenbogen et al. 2000, Coriano et al. 2018). This may be important in some breast cancers secondary to hormone replacement therapy.

III.2. Progesterone receptors

III.2.1. Introduction

Progesterone is a key steroid hormone involved in female reproductive activity. Its effects are mediated by two protein-based progesterone receptors (PR), called PRA and PRB. They originate from a common gene and act as ligand-activated transcription factors to regulate the expression of reproductive target genes (Conneely et al. 2000). Both types of PR are members of the nuclear receptor superfamily and the ratio of the two receptor isoforms varies in reproductive tissues depending on the degree of development, hormonal status and during carcinogenesis (Conneely et al. 2002). PR have a modular structure consisting of distinct functional domains capable of binding ligand, dimerizing hormone-receptor complexes, interacting with hormone-responsive DNA elements, and interacting with co-regulatory proteins required for binding receptors.

Progestogens are a class of compounds that exert progestational activity and include natural progesterone and a variety of synthetic compounds such as medroxyprogesterone acetate, levonorgestrel, megestrol acetate, etc. These substances are administered to postmenopausal women for their protective effect against endometrial hyperplasia that may occur as a consequence of estrogen replacement therapy.

III.2.2. Progesterone receptors – structure, distribution and mechanism of ligand binding

Homologous domains of steroid receptors include a non-conserved variable-length amino-terminal domain, a highly variable TAF-1 domain located near the N-terminal region, a highly conserved DNA-binding domain, and a C-binding domain ligand, moderately conserved. The TAF-1 domain is ligand-independent and required for optimal transcriptional activity, established by protein interactions with transcription factors and co-factor proteins (Lavery et al. 2005).

The action of progesterone and synthetic progestogens, mediated through steroid receptors, is also hampered by the presence of several of their isoforms. The progesterone receptor exists as two isoforms, PR-A and PR-B, transcribed by two promoters of the same gene. The PR-B isoform is longer and more active compared to PR-B. It also contains a functional domain not found in PR-A that binds co-activator proteins to PR-B (Giangrande et al. 2000).

CHAPTER IV. MANAGEMENT STRATEGIES OF THE SYMPTOMATOLOGY AND PATHOLOGY ASSOCIATED WITH MENOPAUSE

IV.1. Management of vasomotor disorders

Under the generic name of vasomotor disorders are included several symptoms such as the feeling of intense heat and sweating, especially at night, which can last between 5 and 10 minutes and can even lead to mood and sleep disturbances requiring medical attention (Blümel et al. 2011). Currently, estrogen replacement therapy alone or combined with progesterone administration is the most common and effective therapeutic strategy applied for the relief of hot flashes and night sweats in menopause. Estrogen hormone replacement therapy can usually reduce the severity and frequency of hot flashes and hot flashes by more than 70% within about a month of continuous use (Al-Safi et al. 2014). Combined sublingual or bioidentical topical preparations for hormone replacement therapy have also been shown to be effective in ameliorating menopausal vasomotor disturbances (Ruiz et al. 2014). Non-hormonal pharmacologic therapies for menopausal vasomotor disorders include selective serotonin reuptake inhibitors, selective norepinephrine reuptake inhibitors, clonidine, and gabapentin (Krause et al. 2015).

IV.2. Management of postmenopausal headache and migraine syndrome

Regarding the treatment for the relief of headache and migraine, this is still controversial and hormone replacement therapy is not considered first-line therapy in postmenopausal headache and migraine. There is no evidence that hormonal treatments for migraine are more effective or safer than non-hormonal treatments. Most hormone treatments for migraine have been tested in case series or small clinical trials in selected populations, which are insufficient to fully establish the balance of harms and benefits. Also, a recent Cochrane report on the use of long-term hormone replacement therapy concluded that "long-term health effects have not been documented"(Edelman et al. 2004). Plants with high phytoestrogen content that are traditionally used to relieve headaches and migraines may also be useful in the holistic treatment of migraines or menopausal headaches (Villella 2016). Venlafaxine is a phenylethylamine derivative that facilitates neurotransmission by blocking the presynaptic reuptake of serotonin (5-hydroxytryptamine, 5-HT) and noradrenaline (norepinephrine). Clinical data from patients with major depression are consistent with the favorable efficacy and tolerability profile of venlafaxine predicted by pharmacodynamic studies (Carboni et al. 2019). Although it is a drug mainly used in the treatment of depression, Venlafaxine has also proven its effectiveness as a therapeutic agent used in migraine and postmenopausal headache.

IV.3. Management of depressive and cognitive disorders during menopause

As depression in the elderly is an important public health problem, a possible correlation between menopause and depression is of major practical importance. There is some evidence that estrogen replacement therapy may be considered for the relief of mild depressive symptoms associated with hot flashes, sleep disturbances, or other menopausal symptoms. However, there are no concrete data from randomized trials to indicate whether estrogen can be used as adjunctive therapy for depressive disorders during the menopausal transition or postmenopause, but the new findings suggest that estrogens may be useful as adjunctive therapy to treatment with serotonin reuptake inhibitors (Birkhäuser 2002).

IV.4. Management of genitourinary syndrome and sexual disorders

Vaginal dryness is a common condition during menopause and in the postmenopausal period and is one of the symptoms of vulvovaginal atrophy or menopausal genitourinary syndrome. The impact of vaginal dryness on interpersonal relationships, quality of life, daily activities and sexual function can be significant, but is often underestimated. Topical estrogen therapy is considered first-line therapy because it is effective and well tolerated for the treatment of moderate to severe symptoms. It acts by restoring the premenopausal vaginal microenvironment, i.e. by thickening the epithelium, by increasing vaginal secretions, by restoring vaginal flora and by lowering vaginal pH. Overall, estrogen therapy decreases vaginal dryness and improves urogenital symptoms (Tadir et al. 2017). Selective estrogen receptor modulators (SERMs) represent a heterogeneous group of nonsteroidal compounds that act as estrogen receptor ligands and have proven efficacy in the treatment of genitourinary syndrome by exerting a mixed action of agonists or antagonists of these receptors , depending on the target tissue (Martinkovich et al. 2014). Ospemifene is the only FDA-approved non-hormonal therapy for dyspareunia caused by menopausal vulvovaginal atrophy (Hill et al. 2016).

Other natural products that have proven effective in maintaining vaginal health include phytoestrogens, which are nonsteroidal plant compounds with the ability to bind to estrogen receptors. Some studies suggest beneficial effects of these compounds on the urogenital sphere as they improve dyspareunia and vaginal dryness. Given the lack of safety data for women with estrogen-sensitive tumors, phytoestrogens should be recommended with caution in all cases (Palacios et al. 2016).

CHAPTER V. THE MECHANISM OF METABOLIC DISORDERS IN POSTMENOPAUSE

V.1. The mechanism of lipid metabolism disorders in post-menopause

Before menopause, women are protected from atherosclerotic cardiovascular disease compared to men of the same age. The production of HDL- and LDL-cholesterol is dependent on endogenous sexual hormone levels and their variations generate a series of imbalances at the level of lipid metabolism. Estrogens can modulate vascular function via estrogen receptors on endothelial cells and also on vascular smooth muscle cells. Estrogens can also cause the release of nitric oxide and prostacyclin, both of which are vasodilator molecules. In addition, they can lead to a reduction in the production of endothelin and angiotensin II, which have a vasoconstrictor effect (Beraldo et al. 2018).

V.1.1. Effects of menopause on body composition

With the onset of menopause, both the distribution of adiposity and the composition of the woman's body undergo a series of changes, with an impact on lipid metabolism. Two patterns were observed for the distribution of adipose tissue, consisting of the accumulation of fat at the gluteo-femoral level as well as the central accumulation of fat at the visceral level. Intra-abdominal distribution is considered an independent risk factor for cardiovascular disease, regardless of the degree of obesity (Beraldo et al. 2018). Android-type adiposity has been associated with increased risk of diabetes, hypertriglyceridemia, hypertension, and other types of cardiovascular disease. Estrogen causes the accumulation of fat at the gluteo-femoral level, and the decrease in the level of this hormone in postmenopause is associated with an increase in visceral adiposity, by deposition at the central level (Ambikairajah et al. 2019). Another factor that seems to be involved in the disposition of

adipose tissue in menopause is the activity of lipoprotein lipase in fat, but the results of studies are still contradictory (Mauriège et al. 2000, Ferrara et al. 2002).

V.1.2. Changes in LDL-, HDL-cholesterol and triglycerides

Studies have shown that in menopausal women the following have been found: LDL-cholesterol, triglycerides and lipoprotein have increased, while HDL-cholesterol levels are very low (Ambikairajah et al. 2019). Although LDL-cholesterol values are not an integral part of metabolic syndrome X, they register increases of approximately 10-20% compared to the premenopausal period and are the main risk factor for atherosclerosis (Matthews et al. 1989). Regarding the level of triglycerides, studies have shown that their serum value increases exponentially with the transition to menopause, the values reaching the highest levels in postmenopause. Also, serum triglycerides are positively correlated with increased abdominal fat content and insulin resistance (Carr et al. 2000).

V.2. Changes in carbohydrate metabolism, insulin resistance and metabolic X syndrome

Two of the most important components of the metabolic syndrome are marked insulin resistance and increased visceral adiposity. The latter is closely related to increased insulin resistance and compensatory hyperinsulinemia, which will ultimately lead to type II diabetes (Pouliot et al. 1992). The pathophysiology of insulin resistance in menopause is complex and long studied. Insulin resistance associated with compensatory hyperinsulinemia will diminish the normal suppression of free fatty acids originating from adipose tissue. In this context, elevated levels of free fatty acids may impair peripheral glucose uptake, increase gluconeogenesis, and reduce hepatic insulin clearance.

V.3. Bone metabolism and structural changes in menopause

During menopause, osteoblast and osteoclast activity is mediated by RANK, RANK ligand (RANKL) as well as osteoprotegerin (Bar-Shavit 2007). In the first phase, RANKL, a molecule produced by osteoblasts, binds to RANK, which is present on the surface of osteoclasts, activating it. Its activation leads to the formation and normal functioning of osteoclasts. Osteoprotegerin is also secreted by osteoblasts and has the role of inhibiting RANKL, while also preventing RANK activation. In this way, the activity of osteoclasts is blocked, and bone remodeling is balanced (Kostenuik 2005). Estrogen is one of the mediators of this process, and the brutal decrease of this hormone in postmenopause produces severe disorders of bone remodeling and leads to increased bone resorption, which will generate osteopenia and finally osteoporosis.

V.4. Sleep metabolism in menopause

There are 2 distinct mechanisms by which menopause affects sleep quality. One is insomnia, which can be considered as an integral part of the symptomatology specific to this period. Another mechanism that participates in affecting the quality of sleep is secondary to sleep apnea or partial obstruction of the upper airways during sleep. Insomnia can often be effectively treated with conventional hormone replacement therapy, while sleep apnea can be treated with progestogens. Many age-related conditions not directly related to menopause also need to be considered when treating postmenopausal sleep disorders (Polo-Kantola et al. 2001).

CHAPTER VI. BENEFITS AND RISKS OF HORMONE REPLACEMENT THERAPY

VI.1. The benefits of hormone replacement therapy

- *Decreases the risk of cardiovascular events*
- *Metabolic effects* - It is worth noting that hormone replacement therapy appears to decrease the risk of type 2 diabetes. However, the impact is insufficient for hormone therapy to be strictly recommended for its prevention (Agarwal et al. 2018). Regarding the variation of serum lipid concentration in patients receiving hormone replacement therapy, a recent study (Gregersen et al. 2019) demonstrated that hormone treatment can reduce plasma concentration of lipoprotein a (Lp(a)), ApoA1, ApoB, total cholesterol, LDL-cholesterol, as well as total cholesterol/HDL-cholesterol and LDL-cholesterol/HDL-cholesterol ratio.
- *Reduces the prevalence of osteoporosis*
- *Reduces vasomotor symptoms and migraines*
- *It improves the symptoms of the genitourinary syndrome*
- *Effects on cognition and dementia* – hormone replacement therapy may improve cognitive function in women with Alzheimer's disease (Zhou et al. 2020)
- *Beneficial effects on sleep disorders*

VI.2. Risks of hormone replacement therapy

- *Increases the risk of breast and endometrial cancers*
- *Increases the risk of deep vein thrombosis and stroke*

CHAPTER VII. PHYTOTHERAPEUTIC COMPOUNDS USED IN PROBLEMS ASSOCIATED WITH MENOPAUSE - DATA FROM CLINICAL STUDIES

According to the most recent studies, in recent decades the interest of medical professionals in the knowledge and use of plant-based medicines, known as phytotherapeutic compounds, has increased significantly. This increased interest in natural compounds may be mainly due to the possible risks of irrational use of allopathic medicines, in addition to their high costs (Balan et al. 2021). The World Health Organization recognizes that approximately 85% of the population of developing countries use plants or plant-based products for the treatment of a wide range of medical conditions (Moga et al. 2021). Therefore, this type of approach deserves increased interest to establish what are the benefits, but also the risks to which patients consuming these types of treatments are exposed (Lopes et al. 2019).

Phytoestrogens are natural nonsteroidal phenolic plant compounds that, due to their molecular structure and size, closely resemble estrogen while exerting similar effects. Flavonoid isoflavones from plants are considered the most potent pseudoestrogenic sources. The main dietary sources of isoflavones are soy and soy products, which mainly contain daidzein and genistein. According to recent data, isoflavones from soy can act as selective modulators of estrogen receptors, which is why they exert a series of beneficial effects on cardiovascular indices: lowering the level of total cholesterol and LDL-cholesterol, of serum triglycerides, decreases endothelial dysfunction. Soy isoflavones have also been shown to prevent bone loss in patients regardless of body weight and treatment period. They

increased bone mineral density in normal weight women and decreased bone resorption in overweight or obese patients. According to the data in the literature, the daily consumption of some soy-based products can significantly reduce the number of hot flashes and sweating episodes in patients in this age group (Imhof et al. 2018).

Cimicifuga racemosa (black cohosh) extract may be useful for the treatment of menopausal symptoms such as hot flashes, heavy sweating, insomnia and anxiety (Mahady et al. 2002). The results of preclinical studies suggest that the effectiveness of black cohosh extracts in relieving menopausal symptoms could be the effect of substances with dopaminergic or serotonergic activity.

Spirulina is a microscopic filamentous phytosynthetic cyanobacterium that grows naturally in highly saline alkaline lakes in Africa, Mexico, the Americas, and Asia. It has been shown that many bioactive peptides extracted from Spirulina possess antimicrobial, antiviral, antitumor, immunomodulatory, antiallergic and antihypertensive properties. It has been reported that consumption of Spirulina could prevent or ameliorate components of the metabolic syndrome so prevalent during menopause (Bobescu et al. 2020).

Dietary supplements based on red clover (Trifolium pratense L.) have received increasing attention from investigators, given their beneficial effects in the treatment of menopausal symptoms, maintaining and/or improving bone and cardiovascular health. In addition, their benign effects on the breast and endometrium have made them valuable therapeutic agents for menopausal women. Currently, clinical evidence supporting the efficacy of isoflavones extracted from semi-purified red clover for ameliorating menopausal vasomotor symptoms or reducing serum LDL-cholesterol levels is limited. In addition, the safety of using these dietary supplements in patients with breast or endometrial cancer is still an area under study. Limited evidence suggests a possible efficacy in maintaining bone health and improving arterial compliance, a risk factor for atherosclerosis (Booth et al. 2006).

Royal jelly is known as a "superfood", a creamy substance, secreted by the mandibular and hypopharyngeal glands of bees, used to feed queen bees throughout their lives and worker bees during the larval stage. Royal jelly improves reproductive health, is an adjuvant in the treatment of neurological diseases and possesses several biological properties, such as: antibacterial, vasodilator, anti-inflammatory, hypotensive, anticarcinogenic, estrogen-like, hypocholesterolemic, hypoglycemic and antioxidant effects. In recent years, royal jelly has been reported as a valuable medicinal agent for healthy aging and longevity (Balan et al. 2020). It has also been shown that the use of this alternative treatment can exert a number of beneficial effects in menopausal women, being a potential substitute for hormone replacement therapy, alone or in combination with other phytotherapeutic compounds.

PERSONAL RESEARCH SECTION

CHAPTER VIII. PURPOSE AND OBJECTIVES OF THE RESEARCH

This study is a prospective observational analysis of the evolution, from a clinical and paraclinical point of view, of women in both postmenopausal period and menopausal transition period, who benefited, or not, of the administration of some dietary supplements known for their effects on the menopausal symptomatology. The main aim of this thesis was to investigate the effectiveness of natural food supplements in improving peri- and post-menopausal symptoms, as well as their influence on cardiovascular risk factors, by comparing two groups treated with natural non-hormonal dietary supplements, with a control group that did not receive any kind of dietary supplement.

Specific objectives:

- Analysis of the evolution of vasomotor symptoms, sleep disorders, sexual disorders, clinical and paraclinical parameters, MENQOL quality of life scores and DASS-21R scores, comparatively, in groups treated with dietary supplements versus the untreated group.
- Determining the level of correlation between MENQOL and DASS-21R scores and other variables such as age, physical exercise, body mass index, number and intensity of hot flashes, wake-up time, number of monthly sexual contacts, in the three groups.
- Determining the level of correlation between salivary cortisol levels collected at 12 pm and wake-up time, daily number of hot flashes, and intensity of hot flashes in the three groups.
- Establishing an optimal therapeutic regimen, based on non-hormonal dietary supplements, for alleviating menopause-associated symptoms.

CHAPTER IX. RESEARCH METHODOLOGY

IX.1. Study design

The research was carried out in the Clinical Hospital of Obstetrics and Gynecology "Dr. Ioan Aurel Sbârcea" from Brașov in the period 2020-2022, and the included patients were selected from among those who addressed the hospital's Ambulatory for complaints specific to the menopausal period and met the inclusion criteria.

The study was designed as an observational, prospective study with a follow-up period of 8 weeks. The study consisted in the enrollment, evaluation and clinical-therapeutic follow-up of three groups of women in the postmenopausal period or menopausal transition period, who complained of symptoms specific to these periods, and who administered non-hormonal dietary supplements for the management of these symptoms or refused to take any type of dietary supplement. The therapy was indicated by their attending physician.

The type of dietary supplements and the doses were indicated by the attending physician, but for an adequate reliability of the results and in order to minimize the differences between groups, we included only the patients who consumed the same type of dietary supplements, with the same daily dosage. The two types of dietary supplements were: a dietary supplement based on fermented soy extract (Femarelle Recharge) and liophilized royal jelly capsules. Each patient underwent two assessments: at enrollment and after 8 weeks. Upon the inclusion in the study, after reading and

signing the informed consent, a personal interview sheet was drawn up for each patient. Approximately 40-60 minutes were allocated to each woman.

All patients participated in two study visits. At the initial visit, the patients answered the questions provided in the personal interview sheet, completed the MENQOL and DASS-21R questionnaires, were weighed, their blood pressure was measured and at the end of the visit a blood sample and a urine sample were collected. In order to collect the saliva for the dosage of salivary cortisol, the patients received two *Salivette* containers together with the instructions for use that were also explained to them verbally, and the collection was to be carried out at 12 pm+/- 30 minutes and the sample was to be brought personally in the morning of the next day. The second Salivette container was used to collect saliva the evening before the second visit. At the second visit, which took place after 8 weeks, in the first part of it the patients answered the questions provided in the personal interview sheet, filled in the MENQOL and DASS-21R questionnaires. Then, their blood pressure was measured and blood and urine samples were collected again, following the procedures from the initial visit.

During the entire period of this study, the norms of ethics and deontology of scientific research were respected, and all the included patients signed an informed consent before the enrollement. The study was approved by the medical ethics committee of the Clinical Hospital "Dr.I.A.Sbârcea" Brașov and by the medical ethics committee of the Faculty of Medicine of Transilvania University Brașov. Also, in this work we did not use personal identification data about the patients, which could reveal their identity.

IX.2. Description of the study groups

Inclusion criteria:

- women aged between 45 and 60 years old
- women able to read and write
- women with menopausal symptoms (minimum 3 hot flashes per day)
- women who were going to start taking a dietary supplement based on fermented soy extract (Femarelle Recharge) as indicated by the attending physician
- women who were going to start taking a dietary supplement based on fermented soy extract (Femarelle Recharge) in asoociation with royal jelly capsules as indicated by the attending physician

Exclusion criteria:

- women with psychiatric pathology
- women with chronic diseases (diabetes, hypertension, etc.)
- women with current or past malignancies
- women with chronic treatments
- women who use or have used in the past hormone replacement therapy or other types of dietary supplements for the relief of menopausal syndrome
- women using drugs that can influence cortisol secretion
- women with clinical signs characteristic for an excess of cortisol (full moon facies, bison nape or skin atrophy)
- women who refused the inclusion in one of the study groups

The control group - It includes women in the menopausal transition or postmenopausal period who consecutively addressed the Clinical Hospital "Dr. I.A. Sbârcea" Brașov for specific menopausal disorders and who refused the administration of dietary supplements at the indication of their attending physician. Patients who did not attend the second visit and those who did not properly collect their saliva samples were excluded from the final statistical analysis. 40 patients were considered for the statistical analysis.

The group treated with a dietary supplement based on fermented soy extract (group S)- It includes women in the menopausal transition or postmenopausal period who consecutively addressed the Clinical Hospital "Dr. I.A. Sbârcea" Brașov for specific menopausal disorders. These patients consumed a dietary supplement based on fermented soy extract (Femarelle Recharge® - 322 mg fermented soy extract per capsule) daily, twice a day, for 8 weeks, at the indication of their attending physician. Patients who interrupted their medication during the follow-up period or who repeatedly missed doses according to the indicated posology, but not due to adverse effects that required stopping the therapy, were excluded from the final statistical analysis. 40 patients were considered for the statistical analysis.

The group treated with a dietary supplement based on fermented soy extract and liophilized royal jelly capsules (group SLM)- It includes women in the menopausal transition or postmenopausal period who consecutively addressed the Clinical Hospital "Dr. I.A. Sbârcea" Brașov for specific menopausal disorders. These patients consumed a dietary supplement based on fermented soy extract (Femarelle Recharge® - 322 mg soy extract per capsule) in association with 1500 mg royal jelly (daily dose) in capsule form, daily, twice a day, for 8 weeks, at the indication of their attending physician. Patients who interrupted their medication during the follow-up period or who repeatedly missed doses according to the indicated posology, but not due to adverse effects that required stopping the therapy, were excluded from the final statistical analysis. 40 patients were considered for the statistical analysis.

IX.3. Research tools

MENQOL questionnaire

The patients received a Romanian version of this questionnaire, which contains 29 items divided into four domains, as follows: vasomotor, psychosocial, physical and sexual domain, each of them having a different number of items. To obtain a total MENQOL score, a seven-point Likert scale applied by the patient for each item was converted to an eight-point scale ranging from 1 to 8. If the woman did not experience that symptom and responded with " no", the score was given 1 point. If the woman experienced the symptom but it was not bothersome at all ("0"), the score was 2. Scores between 3 and 8 corresponded to symptom intensity of "1" to "6" and indicated an increasing intensity of symptomatology. Each domain obtained a final score, represented by the sum of the converted scores of each symptom.

DASS-21R questionnaire

This questionnaire is designed to assess the psychological status of patients and is based on a dimensional conception of psychological disorders. DASS-21R includes 21 items and focuses on three domains: depression, anxiety and stress, being a short and validated version in Romanian of the original DASS questionnaire, which included 42 questions. Each statement was reported in the last week and given one of the following scores: 0- did not happen at all, 1- happened to a certain extent, or only sometimes, 2- happened to a considerable extent, or in quite a lot of the time, 3- happened a lot, or most of the time. Depending on the final score obtained for each domain, the symptomatology was rated with one of the following: "normal", "medium", "moderate", "severe" and "extremely severe".

Interview questionnaire

The interview questionnaire included the following information: patient's age, residence, tobacco use, educational level, reproductive stage (menopausal transition or postmenopausal period), mode of entering menopause (natural or surgically induced), physical exercise practice, daily number and intensity of hot flashes, waking time. This questionnaire was initially tested on 10 women to determine its comprehensibility and ease of use. None of these women were included in any of the three study groups.

IX.4. Variables

The dependent variables investigated in order to evaluate the effectiveness of the therapy with dietary supplements were: the scores resulting from the application of the MENQOL quality of life questionnaire (total score, vasomotor domain score, psychosocial domain score, physical domain score, sexual domain score), the resulting scores following the application of the DASS-21R questionnaire (total score, depression dimension score, anxiety dimension score, stress dimension score), fasting glycemia, HDL-cholesterol, LDL-cholesterol, total cholesterol, triglycerides, ESR, CRP, ALT, AST, salivary cortisol (collected at 12pm), systolic and diastolic blood pressure, urine culture, number of daily hot flashes, intensity of hot flashes reflected by a general symptomatology score, number of monthly sexual contacts and time of spontaneous awakening in the morning. All these variables were recorded at both assessments.

Demographic and clinical covariates were used in multivariate analyzes to adjust for demographic and clinical factors, respectively, that might be correlated with the dependent variables. The demographic variables monitored were age, residence (rural or urban), educational level, type of menopause (menopausal transition or postmenopause), mode of entry into menopause (natural or surgically induced), tobacco use, physical exercises performance, type of diet, body mass index (BMI) and BMI category (normal weight, overweight, obesity grade 1, obesity grade 2, obesity grade 3).

IX.5. Methods of statistical analysis

For the statistical analysis of the data we used the IBM SPSS Statistics for Windows software, Version 26.0. Armonk, NY: IBM Corp, and the database was created using Microsoft Excel. We analyzed continuous variables for normality and then expressed them as mean±standard deviation, modal value, minimum and maximum, and plotted the frequency distribution using histograms. The normality

of the distributions of continuous variables was tested using the Kolmogorov–Smirnov test, while the equality of variances was tested using Levene's test.

We analyzed the association between the categorical variables using the cross table and the $\chi 2$ (chi-square) test. We calculated the chi-square value and the number of degrees of freedom, according to which we determined the value of p, representing the level of statistical significance.

The degree of correlation between the studied parameters was assessed by calculating the Pearson correlation coefficient (r), also known as the linear correlation coefficient. It can take values between -1 and 1 and reflects the degree of correlation between two variables. Depending on the value of r, correlations can be: nule, negative or positive.

The significance of differences between two groups was assessed using Student's t test. To assess the statistical significance of the difference between percentages, we used Pearson's chi-square or Fisher's exact test. For the analysis of more than two groups, the One-Way ANOVA method was applied for continuous variables. To compare the averages of the parameters between paired samples, we used the t-test for dependent samples (in the case of continuous variables), respectively the Wilcoxon test (in the case of ordinal variables). We thus determined the value of p, representing the level of statistical significance. $p<0.05$ was considered statistically significant.

CHAPTER X. RESULTS

X.1. Characterization of the studied groups at baseline

X.1.1. General characteristics of the groups

Table X.1. General characteristics of the groups

	Control group	Group S	Group SLM	p
Age	51.67±4.45	50.67±3.48	50±3.47	0.267
Residency				
Rural	12 (30%)	14 (35%)	14 (35%)	0.875
Urban	28 (70%)	26 (65%)	26 (65%)	
Tobacco use				
Smokers	12 (30%)	13 (32.5%)	9 (22.5%)	0.354
Non-smokers	28 (70%)	27 (67.5%)	31 (77.5%)	
Education				
Middle school	7 (17.5%)	9 (22.5%)	4 (10%)	0.437
Vocational school	9 (22.5%)	4 (10%)	5 (12.5%)	
High school	14 (35%)	19 (47.5%)	17 (42.5%)	
Higher education	10 (25%)	9 (22.5%)	14 (35%)	
Reproductive period				
Menopausal transition	13 (32.5%)	14 (35%)	17 (42.5%)	0.362
Post-menopause	27 (67.5%)	26 (65%)	23 (57.5%)	
Menopausal onset				
Natural	29 (72.5%)	33 (82.5%)	32 (80%)	0.346
Surgical induced menopause	11 (27.5%)	7 (17.5%)	8 (20%)	
BMI	28.55±5.67	28.54±5.54	28.21±5.31	0.953
BMI category				
Normal weight	13 (32.5%)	13 (32.5%)	13 (32.5%)	0.656
Over weight	11 (27.5%)	14 (35.0%)	18 (45.0%)	
Grade 1 obesity	12 (30.0%)	9 (22.5%)	6 (15.0%)	
Grade 2 obesity	0 (0.0%)	1 (2.5%)	0 (0.0%)	
Grade 3 obesity	4 (10.0%)	3 (7.5%)	3 (7.5%)	
Phisical activity performance				
No physical exercices	33 (82.5%)	36 (90.0%)	30 (75.0%)	0.205
> 3 times/ week	1 (2.5%)	0 (0.0%)	0 (0.0%)	
1-3 times/week	1 (2.5%)	2 (5.0%)	6 (15.0%)	
1-3 times/month	5 (12.5%)	2 (5.0%)	4 (10.0%)	
Type of diet				
Normal diet	34 (85.0%)	32 (82.5%)	32 (82.5%)	0.885
Vegetarian diet	2 (5.0%)	3 (7.5%)	4 (10.0%)	
Ovo-lacto-vegetarian diet	3 (7.5%)	4 (10.0%)	2 (5.0%)	
Diery-free diet	1 (2.5%)	0 (0.0%)	1 (2.5%)	

Group S- the group treated with dietary supplements based on fermented soy extract ;
Group SLM- the group treated with dietary supplements based on fermented soy extract and liophilized royal jelly capsules

X.1.2. DASS-21R score

In the control sample the mean total DASS-21R score was 18.3 points (SD 12.68). In group S, the mean total DASS-21R score was 17.5 points (SD 11.81). In the third group, the mean value of the total DASS-21R score was 20.42 points (SD 10.7). The differences recorded between the three samples were not statistically significant regarding the total DASS-21R score (p=0.518). Likewise, the differences recorded between the average values of the depression, anxiety and stress directions subscores were statistically insignificant (p= 0.702, p= 0.862, respectively p= 0.124).

X.1.3. MENQOL score

Analyzing the total MENQOL scores in the control sample at the first study visit we observed a mean value of 113.4 points (SD 26.58). In group S, the mean baseline value of the total MENQOL score was 113.52 points (SD 39.05). In the third group, the mean total MENQOL score was 111.3 points (SD 26.17). The analysis of the variance of the averages of the total MENQOL score and its subdomains scores did not reveal statistically significant differences between the three study groups at the initial visit (total MENQOL score: p= 0.938; vaso-motor domain score: p= 0.741; psychosomatic domain score: p = 0.918; physical domain score: p= 0.934; sexual domain score: p= 0.536).

X.1.4. Clinical and paraclinical characteristics

- Fasting glucose level

The fasting glucose levels in the control group recorded an average value of 106.35 mg/dL (SD 25.49). In group S, a mean value of 107.75 mg/dL (SD 15.39) was obtained. For the SLM group, the mean fasting glycemia value at baseline was 107.25 mg/dL (SD 12.53). The variance analysis of the means did not reveal statistically significant differences between the three study groups at baseline (p= 0.944).

- HDL-cholesterol

The mean value of HDL-cholesterol in the control group was 46.125 mg/dL (SD 12.94). In group S, the mean value of pre-treatment HDL-cholesterol was 42.35 mg/dL (SD 9.2), while in the SLM group the mean value was 45.47 mg/dL (SD 9.45). The variance analysis of the means did not reveal statistically significant differences between the three study groups at the initial evaluation (p= 0.252).

- LDL-cholesterol

The mean baseline value of LDL-cholesterol in the control group was 167.28 mg/dL (SD 48.18). In group S, the mean value of LDL-cholesterol was 165.13 mg/dL (SD 41.88). At enrollment, mean LDL-cholesterol in the group SLM was 159.73 mg/dL (SD 27.12). The variance analysis of the means did not reveal statistically significant differences between the three study groups at the initial visit (p= 0.687).

- Total cholesterol

Analyzing the total cholesterol values of the control sample at baseline, we observed an average value of 218.25 mg/dL (SD 50.40 mg/dL). In group S, the mean value of total cholesterol was 230.77 mg/dL (SD 41.79) and in the group SLM it was 218.25 mg/dL (SD 50.40). The variance analysis of the means did not reveal statistically significant differences between the three study groups at baseline (p= 0.402).

- Triglycerides

Regarding the triglyceride (TG) values, the mean value obtained at the time of inclusion in the control sample was 170.57 mg/dL (SD 76.56). In group S, the mean TG values obtained before therapy was 173.15 mg/dL (SD 66.23) and in the group SLM it was 185.15 mg/dL (SD 49.17). The variance analysis of the means did not reveal statistically significant differences between the three study groups at baseline (p= 0.565).

- Aminotransferases

Analyzing the ALT and AST values in the control sample at baseline we observed that the mean values were 23.55 IU/L (SD 8.9) and 20.67 IU/L (SD 7.32), respectively. In group S, the mean values of ALT and AST were 23.57 IU/L (SD 6.69) and 20.4 IU/L (SD 5.76), respectively. In the group SLM we observed average values of 24.97 IU/L (SD 6.62), respectively 22.42 IU/L (SD 5.79). The analysis of the variance of the means did not reveal statistically significant differences between the three study groups at the initial visit (p= 0.640 respectively p= 0.315).

- Blood sedimentation rate (ESR) and C-reactive protein (CRP)

The mean value of the ESR in the control group was 17.77 mm/h (SD 8.33), and the mean value of the PCR in the control group was 0.66 mg/L (SD 1.05). In group S, the mean value of ESR was 19.6 mm/h (SD 4.82 mm/h), while the mean value of PCR was 1.11 mg/L (SD 1.78). In the SLM sample, the mean ESR value at the first visit was 19.22 mm/h (SD 4.55) and the mean PCR value in this group was 0.61 mg/L (SD 1.10).

The analysis of the variance of the means did not reveal statistically significant differences between the three study groups at the initial visit (p= 0378, respectively p= 0.196).

- Salivary cortisol (collected at 12 pm)

Analyzing the salivary cortisol values (collected at 12 pm) at the first study visit in the control group, we observed an average value of 9.17 nmol/L (SD 8.66). In group S, the mean baseline value of salivary cortisol (collected at 12 pm) was 9.38 nmol/L (SD 6.18). In the third sample, salivary cortisol registered a mean value of 9.56 nmol/L. The variance analysis of the means did not reveal statistically significant differences between the three study groups at the initial evaluation (p=0.964).

- Systolic Blood Pressure (SBP)

Analyzing the systolic blood pressure (SBP) values in the control sample at the initial visit, we observed that its average value was 121.22 mmHg (SD 12.26). In the S sample, the mean SBP was 123.6 mmHg (SD 17.92). For the third group, we obtained an average SBP at inclusion of 118.82 mmHg (SD 12.69). The variance analysis of the means did not reveal statistically significant differences between the three study groups at the initial visit (p=0.343).

- Diastolic Blood Pressure (DBP)

Diastolic blood pressure (DBP) in the control sample had a mean value of 82.9 mmHg (SD 12.04). In the group S, the mean DBP at the time of inclusion was 82.9 mmHg (SD 9.22). For the third group, we obtained an average DBP at baseline of 80.75 mmHg (SD 3.95). The variance analysis of the means did not reveal statistically significant differences between the three study groups at the initial visit (p= 0.474).

- Urine culture

At baseline, the S group had the highest percentage of sterile urine cultures (80%), followed by the SLM group (70%) and the control group (65%). The SLM group had the highest percentage of positive urine cultures with E. coli (25%), followed by the control group (22.5%) and the S group (12.5%). Urinary tract infections with Klebsiella as a pathogen were the most common in the control group (7.5%), in the S and SLM groups each 2.5% of patients presented a positive urine culture for this bacterium. Entroccocus faecalis was detected in 2.5% of the cases cases from the S group, while Group B Streptococcus was found in 5% of control group urine cultures and in 2.5% of S and SLM group urine cultures. Analysis of variance did not identify statistically significant differences between the three samples (p= 0.591).

X.1.5. Vasomotor symptoms, sleep and sexual disturbances

- Number of daily hot flashes

In the control group, the women reported at the first study visit an average number of 7.75 daily hot flashes (SD 2.13). In the S group, the participants reported an average of 8.75 daily hot flashes (SD 3.82) at baseline. In the last sample, the mean number of daily hot flashes was 7.17 (SD 2.18). The

analysis of the variance of the means did not reveal statistically significant differences between the three study groups at the initial visit (p= 0.189).

- The intensity of hot flashes

Depending on the intensity of the hot flashes, the patients were asked to choose one of the following categories from the interview questionnaire: extremely weak, very weak, weak, moderate, strong, very strong and extremely strong hot flashes. In the control group, the highest percentage of participants were included in the category of extremely strong hot flashes (27.5%), followed by 20% of cases with very strong hot flashes and 17.5% of cases with strong hot flashes. In group S, 42.5% of cases reported hot flashes of moderate intensity, followed by 32.5% of participants with strong hot flashes. In the SLM group, 37.5% of participants reported experiencing very strong hot flashes, while 32.5% of cases included in this group had strong hot flashes. No statistically significant differences between groups were recorded (p=0.816).

- Waking time

In the control sample the patients reported a mean time of spontaneous awakening in the morning of 5.52 (SD 1.19). Two of the 40 patients reported a waking time of 3, while the maximum time of spontaneous awakening was 8. In the S group, the mean time of spontaneous awakening was 5.65 (SD 1.33), with extremes similar to the control group. In the SLM group, the mean time of awakening was 5.97 (SD 1.16), with extremes at 4 o'clock and 8 o'clock. The differences between the three groups were not statistically significant (p= 0.267)

- The number of monthly sexual contacts

In the control group we obtained an average of approximately 1.85 sexual contacts/month (SD 2.13), with a minimum of 0 and a maximum of 8. In the S group, the average was 1.45 sexual contacts/month (SD 1.66), with a a minimum of 0 and a maximum of 7, while in the SLM group they reported an average of 1.52 sexual contacts/month, with a minimum of 0 and a maximum of 6. In both groups treated with dietary supplements, the highest percentages of cases have reported 0 and 1 monthly sexual contact, respectively. The differences between the groups were not statistically significant regarding the average number of monthly sexual contacts (p= 0.567).

X.2. Characteristics of the studied groups at 8 weeks

X.2.1. DASS-21R score

In the control group, after 8 weeks from the initial visit, the mean total DASS-21R score was 18.3 points (SD 11.64). The minimum score was 4 points, while the maximum DASS-21R total score was 50 points, recording an increase of 9 points compared to the initial visit. In group S, the mean total DASS-21R score was 14.05 points (SD 11.23). The minimum score was 2 points, while the maximum score

was 51 points. In the SLM group, the mean value of the total DASS-21R score after 8 weeks of treatment was 16.05 points (SD 10.7), recording a decrease of 4.37 points compared to the baseline value. The minimum score obtained was 2 points, while the maximum DASS-21R total score was 40 points. Analysis of the variance of the means in the three samples did not identify statistically significant differences after 8 weeks from baseline, regarding the values of the total DASS-21R score (p=0.403) and the subscores of its directions: depression (p=0.845), anxiety (p=0.537) and stress (p= 0.243).

X.2.2. MENQOL score

Analyzing the total MENQOL score after 8 weeks, in the control group we observed a mean value of 114.9 points (SD 39.13), recording an increase of 1.5 points compared to the baseline. In the S group, the mean total MENQOL score after 8 weeks of administration of a dietary supplement with fermented soy extract was 100.52 points (SD 38.56), 12.98 points lower than the baseline value. In the SLM group, the mean total MENQOL scores after the administration of the fermented soy extract in associations with royal jelly capsules was 80.07 points, 31.23 points lower than the baseline.

Analysis of variance of the total MENQOL score means identified statistically significant differences between the three groups after 8 weeks (p<0.001). Also, the average scores of the vaso-motor, psychosomatic, physical and sexual subdomains showed statistically significant differences between the three groups at the second evaluation (p<0.001).

X.2.3. Clinical and paraclinical characteristics

- Fasting glucose level

At the second assessment, the fasting blood glucose values of the patients in the control sample recorded an average value of 104.75 mg/dL (SD 21.56 mg/dL). The mean value of 100.05 mg/dL (SD 18.7) was obtained in group S. For the group SLM, the mean fasting blood glucose value after treatment was 94.72 mg/dL (SD 16.23). The differences recorded between the three samples after 8 weeks of follow-up revealed statistically significant differences (p=0.02).

- HDL-cholesterol

At the second evaluation, the mean HDL-cholesterol value in the control group was 46,175 mg/dL (SD 12.52 mg/dL). For group S, the mean value of HDL-cholesterol after 8 weeks of administration of the dietary supplement based on fermented soy extract was 43.55 mg/dL (SD 9.86 mg/dL). For the SLM group, the mean post-treatment HDL-cholesterol value was 56.37 mg/dL (SD 11.5). The differences between the mean values of HDL-cholesterol in the three study samples after 8 weeks from inclusion were statistically significant (p<0.001).

- LDL-cholesterol

The mean value of LDL-cholesterol in the control sample was 168.125 mg/dL (SD 45.44 mg/dL). For group S, the mean value of LDL-cholesterol after 8 weeks of administration of the dietary supplement based on fermented soy extract, was 148.77 mg/dL (SD 40.80 mg/dL). For the SLM group, the mean post-treatment LDL-cholesterol value was 109.35 mg/dL (SD 25.51). The differences recorded between the mean values of LDL-cholesterol in the three study samples after 8 weeks from inclusion were statistically significant ($p<0.001$).

- Total cholesterol

The mean value of total cholesterol in the control group at 8 weeks from baseline was 215.4 mg/dL (SD 46.94 mg/dL). In the group S, the mean value of total cholesterol was 194.2 mg/dL (SD 39.32) and in the group SLM, the mean value of total cholesterol after the administration of royal jelly capsules in association with fermented soy extract was 165.35 mg/dL (SD 21.93). Analyzing the differences between the mean values of total cholesterol at the second evaluation we observed that they were statistically significant between the three study samples ($p<0.001$).

- Triglycerides

At the second assessment, the mean TG value in the control group was 168.1 mg/dL (SD 69.94). For group S, the mean value of TG was 159.35 mg/dL (SD 68.28), while in the SLM group the mean value of TG was 134.72 mg/dL (SD 41.43). The differences between the mean values of TG in the three samples, at 8 weeks after the inclusion in the study, were statistically significant ($p= 0.04$).

- Aminotransferases

At the second assessment, the mean values of ALT and AST in the control group were 23.2 IU/L (SD 7.32) and 20.92 IU/L (SD 7.09), respectively. In group S, the mean values of ALT and AST were 23.05 IU/L (SD 6.67) and 20.63 IU/L (SD 5.79), respectively. In the SLM group we observed mean values of 24.25 IU/L (SD 6.35), respectively 21.1 IU/L (SD 5.53) for ALT and AST. The variance analysis of the means did not reveal statistically significant differences between the three study groups after 8 weeks from baseline ($p= 0.489$, respectively $p= 0.954$).

- Blood sedimentation rate (ESR) and C-reactive protein

At the second study visit, the mean ESR in the control group was 17.55 mm/h (SD 6.09) mm/h. The mean CRP value in this sample was 0.41 mg/L (SD 0.71). After 8 weeks of administration of a dietary supplement based on fermented soy extract, the mean ESR value was 18.45 mm/h (SD 5.14) and the mean CRP value in this sample was 1.16 mg/L (SD 1.82). In the third study group, after 8 weeks of administration of fermented soy dietary supplement in association with lyophilized royal jelly capsules, the mean value of the ESR was 18.85 mm/h (SD 4.89), and the mean PCR value was 0.63 mg/L (SD

1.15). The analysis of differences between the means of ESR and CRP between the three groups after 8 weeks from baseline were not statistically significant (p= 0.597, respectively p= 0.854).

- Salivary cortisol (collected at 12 pm)

After the second assessment we found that the mean value of salivary cortisol in the control sample was 9.16 nmol/L (SD 8.74) . Analyzing post-treatment salivary cortisol values in the S group, we observed that the mean value of salivary cortisol in this group was 4.73 nmol/L (SD 6.87). Compared to the control group, the values obtained in the case of group S, after 8 weeks of administration of a dietary supplement with fermented soy extract, are significantly lower. After treatment, the mean value of salivary cortisol in the SLM group was 2.29 nmol/L (SD 1.28 nmol/L). Compared to the control group and the S group, the values obtained in the case of the SLM group, after 8 weeks of treatment were significantly lower. Analysis of variance of the means revealed statistically significant differences between the three samples after 8 weeks regarding the values of salivary cortisol (p=0.001)

- Systolic blood pressure

On the second evaluation, the mean value of SBP in the control group was 120.45 mmHg (SD 10.52). The mean value of SBP in group S after therapy was 120.75 mmHg (SD 18.59) mmHg and in the SLM group the mean value of SBP was 115.75 mmHg (SD 10.66) Analyzing the variation of SBP averages between the three samples after 8 weeks from inclusion we noticed that the differences had a weak statistical significance (p=0.05).

- Diastolic blood pressure

The mean value of DBP in the control group was 83.125 mmHg (SD 10.06). The mean value of SBP in group S after the treatment was 81.02 mmHg (SD 7.01) mmHg. After 8 weeks of administration of royal jelly capsules, in the SLM group the mean value of DBP was 78.62 mmHg (SD 4.95). Analysis of the variance of the means revealed a weak statistically significant difference between the three groups (p=0.04).

- Urine culture

After 8 weeks of follow-up, the results of the urine culture of the patients in the control group did not change significantly compared to the results obtained at baseline. 62.5% of them had a sterile urine culture, while the number of urinary tract infections with Escherichia coli increased insignificantly (25%). In the S and SLM groups, 80% and respectively, 87.5% of the participants had sterile urine cultures after treatment, while 12.5% each had urinary infections with Escherichia coli. Enteroccoccus faecalis was detected in 2.5% of the cases of group S. 5% of the participants from the control group and 2.5% of those of the S group had a urinary tract infection with Group B Streptococcus at the follow-up visit. The

differences between the urine culture results in the three samples were statistically insignificant after 8 weeks of follow-up (p=0.108).

X.2.4. Vasomotor symptoms, sleep and sexual disturbances

- Number of daily hot flashes

After 8 weeks from baseline, the mean number of daily hot flashes reported in the control group was 7.82 (SD 4.98). In the sample that received a dietary supplement based on fermented soy extract, the average number of daily hot flashes obtained after treatment was 5.52 (SD 3.79). In the group treated with royal jelly capsules in combination with the dietary supplement based on fermented soy extract, the mean daily number of hot flashes after treatment was 2.85 (SD 1.27). The differences recorded between the three samples regarding the mean number of daily hot flashes were statistically significant after 8 weeks of follow-up (p<0.001).

- The intensity of hot flashes

After 8 weeks, 25% of cases in the control group declared that the intensity of hot flashes was extremely strong, followed by a percentage of 22.5% who declared that they had mild intensity hot flashes. Furthermore, the distribution of patients in this group according to the intensity of hot flashes remains varied, with significant percentages in the categories of very strong (20%) and strong (17.5%) intensity. Group S had a significant percentage of participants in the category of moderate intensity of hot flashes (40%), but also a small percentage in the categories of extremely weak (5%) and extremely strong (5%) intensity. Groups S and SLM presented the highest percentages of participants in the category of moderate intensity of hot flashes, both having 40% of cases. Analysis of variance revealed statistically significant differences between the three samples regarding the intensity of hot flashes after 8 weeks from the inclusion in the study (p<0.001).

- Waking time

On the second visit, the mean waking time in the control group was 5.62 am (SD 1.19). After taking dietary supplements based on fermented soy extract, the average time of awakening was 6.4 (SD 1.33). In the third sample, the mean post-treatment waking time was 6.85 am (SD 1.02). The variation of the average hour of spontaneous awakening in the morning between the three study samples registered statistically significant values (p=0.004).

- The number of monthly sexual contacts

After 8 weeks from baseline, the mean number of monthly sexual contacts declared by the patients in the control group was 1.72 (SD 1.99). 32.5% of the cases reported the complete absence of monthly sexual contacts, 30% of the cases reported only one sexual contact/month, while only 2.5% of the cases reported 8 monthly sexual contacts. In group S, the average of the monthly intercourses was 1.4 (SD

1.42). In the last group, the monthly mean was 1.67 (SD 1.55). The variation in the average number of monthly sexual contacts after 8 weeks from the inclusion did not show statistically significant differences between the three study groups according to the analysis of variance of the means (p= 0.403).

X. 3. Treatment efficiency

X.3.1. Evolution of DASS-21R score, MENQOL score, clinical and paraclinical parameters, vasomotor symptoms, sleep and sexual disorders within each study group

The results obtained after the initial evaluation and those obtained after 8 weeks of follow-up, for each group, were analyzed comparatively, in order to observe their evolution after the treatment.

In the control group, in which the patients did not receive any kind of treatment, almost all the analyzed parameters remained constant during the follow-up period. No statistically significant variations between the baseline and final values were observed. Regarding the mean values of total cholesterol, in this group we observed a slight decrease after the 8 weeks of follow-up, but statistically significant (p=0.039), despite the fact that the patients did not follow any treatment. Also, the score of the sexual subdomain of the MENQOL questionnaire registered a slight, but highly statistically significant increase, suggesting that the lack of treatment in these patients worsened the sexual disorders.

In group S, in which the patients received a dietary supplement based on fermented soy extract, there were multiple favorable and statistically significant variations of the analyzed parameters. Except for the values of ESR, CRP, aminotransferases, diastolic blood pressure, the number of monthly sexual contacts and the result of urine culture, all other analyzed variables showed post-treatment improvements. The number of sexual contacts remained constant, and the rate of urinary tract infections was not improved after the administration of the dietary supplement based on fermented soyb extract. Also, the values of transaminases, inflammatory tests and DBP did not undergo significant changes.

Regarding the third study group, the SLM group, the association between the dietary supplement based on fermented soy extract and the royal jelly capsules had a beneficial effect on the psychological status of these participants, as reflected by the DASS-21R score, on the quality of life, reflected by the MENQOL score, on vasomotor symptoms, sleep and sexual disorders and on the values of clinical and paraclinical parameters. The values of transaminases and inflammatory markers remained approximately constant after the treatment, with no statistically significant differences between the two assessments. The number of urinary tract infections was significantly reduced in this group after 8 weeks of treatment.

X.3.2. Comparative analysis of DASS-21R score, MENQOL score, clinical and paraclinical parameters values, vasomotor symptoms, sleep and sexual disorders among the three groups after the treatment

To establish which of the two alternative therapy schemes had a greater effectiveness in improving the values of the presented variables, the post-treatment results in the S group and the SLM group were compared. Patients who received dietary supplements based on fermented soy extract in association with royal jelly capsules had a lower MENQOL score, indicating a more significant increase of the quality of life compared to those who did not receive royal jelly. Also, the scores of the vasomotor, psychosomatic and physical subdomains were improved in the SLM group, without significant differences between the scores of the sexual subdomain. Also, the treatment that included both fermented soy extract and royal jelly caused a more important decrease in the number of daily hot flashes, a decrease in the values of LDL-cholesterol, total cholesterol, TG, salivary cortisol and an increase in HDL-cholesterol level compared to the sole use of the dietary supplement based on fermented soy extract.

In comparison with the control group, the patients who received treatment with a dietary supplement based on fermented soy extract obtained better values of the MENQOL score and the vasomotor subdomain score, presented a reduced number of daily hot flashes with lower intensity. Also, post-treatment salivary cortisol, LDL-cholesterol and total cholesterol values were significantly improved in the S group compared to the control group.

Compared to the control group, the patients included in the SLM group had significantly lower values of the MENQOL scores, as well as of all its subdomains scores, suggesting an increase in the quality of life of the women in this group. At the same time, the daily number and intensity of hot flashes recorded statistically significant decrease after the treatment, and the time of spontaneous awakening during the morning increased. The patients who received these dietary supplements had significantly better values of fasting blood glucose, HDL-cholesterol, LDL-cholesterol, total cholesterol, TG and salivary cortisol. SBP and DBP values were also improved in these patients, and the number of urinary infections was lower.

X. 4. Side effects

Throughout the study period, the patients were closely monitored for the early detection of possible side effects arising from the administration of dietary supplements based on fermented soy extract and lyophilized royal jelly capsules. One of the patients included in group S complained of the intensification of vasomotor symptoms and the increase of headache intensity, which is why the administration of the treatment was stopped early. This case was singular. 92.5% of cases reported no side effects, while 5% reported mild headache. In the SLM group, 97.5% of the cases did not show any side effect, while 2.5% of the cases complained of a mild, transient skin rash.

X.5. Statistical correlations

The degree of correlation between the DASS-21 score, MENQOL score, vasomotor symptoms, salivary cortisol levels, wake-up time, and the covariates taken into account was investigated by calculating the Spearman correlation coefficient in order to observe whether there exist, and how a possible co-dependence manifests between them.

According to the Pearson correlation coefficients, we obtained the following results:

- Age and MENQOL score – weak direct correlation in SLM group, statistically significant at both visits.
- Age and DASS-21R score- there is no statistical correlation.
- Physical activity performance and MENQOL score - in the control group and in the SLM group, the MENQOL score negatively correlated with the frequency of physical exercises performance at both study visits.
- Physical activity performance and DASS-21R score- no statistical correlation in either group.
- BMI and MENQOL score- positive correlation in all study groups, statistically significant, at both study visits.
- BMI and DASS-21R score- statistically significant high positive correlation for the S group at both visits. For the control group and the SLM group we did not observe a correlation between the DASS-21R score values and the BMI values.
- Daily number of hot flashes and MENQOL score - positive correlation in all study groups at both visits, statistically significant.
- Daily number of hot flashes and DASS-21R score - statistically significant positive correlation in all study groups at both visits.
- Hot flashes intensity and MENQOL score- positive correlation in the control group and the S group, statistically significant.
- Hot flashes intensity and DASS-21R score - statistically significant positive correlations of moderate intensity, only in the control group and the S group at both study visits. In the SLM group there was no correlation between these two variables.
- Waking time and MENQOL score- statistically significant, negative and moderate correlations for each study group at both visits.
- Waking time and DASS-21R score- statistically significant, negative and moderate, respectively strong correlations for the control group and for the S group. Within the SLM group, no correlation was found.
- The number of monthly sexual contacts and MENQOL score - negative correlation in all groups at both study visits, statistically significant.
- The number of monthly sexual contacts and DASS-21R score - weak and statistically significant negative correlation in the control group and the SLM group at the first visit. In the case of the second visit, statistically significant, negative and weak correlations are found only for the S and SLM groups.
- Salivary cortisol (collected at 12 pm) levels and the number of daily hot flashes - strong positive correlation in all study groups at both visits, statistically significant.
- Salivary cortisol (collected at 12 pm) levels and hot flashes intensity - statistically significant strong positive correlation in all study groups at both visits.

- Salivary cortisol (collected at 12 pm) levels and waking time - statistically significant, strong negative correlation in all groups at both study visits.

- Daily number of hot flashes and waking time - statistically significant, strong negative correlation in all groups at both study visits.

- Hot flashes intensity and waking time - strong negative correlation in all groups at both study visits and statistically significant.

CHAPTER XII. DISCUSSIONS

In this prospective observational research study, we compared the results obtained following the analysis of some characteristics of three categories of patients: those who received a dietary supplement based on fermented soy extract (Femarelle Recharge®) as indicated by the attending physician, those who received Femarelle Recharge® in association with lyophilized royal jelly capsules and those who refused the intake of any type of dietary supplement in order to improve the symptoms specific to the menopausal transition and postmenopause.

Regarding the MENQOL score, there were no statistically significant differences between the three study cohorts at baseline. After 8 weeks of follow-up, comparing the results from the control group and the S group, we observed a significant improvement in the MENQOL score and the score of the vasomotor domain in group S. At the same time, comparing the values of the MENQOL score and its subdomains scores, obtained at the second evaluation in the control group with those obtained in the SLM group after 8 weeks of treatment, we observed a statistically significant improvement for all of them in the SLM group. For both the S group and the SLM group, the differences between the initial and the final values of the total MENQOL score and the scores of each domain were statistically significant. Comparing the post-treatment values in the two groups treated with dietary supplements, we observed a superiority of the association between dietary supplements with fermented soy extracts and royal jelly versus solitary administration of soy supplements on the values of the total MENQOL score and the subscores of the vasomotor, psychosomatic, physical and sexual domains.

The effects of soy isoflavones on menopausal symptoms and quality of life were also illustrated in a meta-analysis, which included all randomized clinical trials conducted on this topic up to September 2023 (Gencturk et al. 2024). The results revealed that soy isoflavones had no effect on vasomotor, psychosocial, physical and sexual symptoms, did not cause significant changes in the MENQOL score, but reduced the level of depression. In our study, in none of the groups treated with dietary supplements the depression was not influenced by the treatment.

Regarding the effects of royal jelly on menopausal symptoms and quality of life in postmenopausal women, the studies in the literature are insufficient in order to formulate a clear conclusion. However, according to a study conducted by Sharif et. al (Sharif et al. 2019) which included 200 women between 45 and 60 years old, who received 1000 mg of royal jelly daily for 2 months, the consumption of this dietary supplement significantly improved menopausal symptoms and patients' quality of life. In the control group, this effect was insignificant.

In this study, the evolution of some clinical and paraclinical parameters was analyzed over a period of 8 weeks, both in the groups that received dietary supplements and in the control group. These parameters were fasting blood glucose, HDL-cholesterol, LDL-cholesterol, total cholesterol, TG, ESR, CRP, ALT, AST, salivary cortisol (collected at 12 pm) and urine culture. The initial values, recorded at the first study visit, were homogeneous in the three groups, without statistically significant differences.

Comparing the control group and the S group post-treatment, a statistically significant difference was observed in the values of LDL-cholesterol, total cholesterol and salivary cortisol, which were improved in group S.

Comparing these parameters in the control group and the SLM group, after 8 weeks of administration of a dietary supplement based on fermented soy extract in association with liophilized royal jelly capsules, the values of fasting blood glucose, HDL-cholesterol, LDL-cholesterol, total cholesterol, TG and salivary cortisol were significantly improved in women who received the treatment. Also, compared to the control group, there were significantly fewer urinary tract infections in this group.

We also analyzed the values of these biological parameters within each study group, at the initial visit and after 8 weeks of treatment. In the control group, the values of total cholesterol were significantly lower at the second visit. Both in the S group and in the SLM group, after 8 weeks of treatment, fasting blood glucose, HDL-cholesterol, LDL-cholesterol, total cholesterol, TG and salivary cortisol recorded significantly better values. In addition, in the SLM group we also observed a decrease in the incidence of urinary infections compared to the baseline results.

A meta-analysis realised by Yang et al. (Yang et al. 2023), which included the results of randomized clinical trials conducted between 2000 and 2023, concluded that doses below 80 mg/day or above 80 mg per day of isoflavones extracted from soy significantly decreased serum TG values and increased HDL-cholesterol values. The duration of treatment for a significant improvement in TG values exceeded 24 weeks, but changes in HDL-cholesterol values were observed after less than 24 weeks of administration. These observations were valid for patients under the age of 65. Therefore, the effectiveness of soy isoflavones in the management of lipid metabolism disorders occurring during menopause, which we observed in the present study, is also supported by the results of this meta-analysis.

The results of our study were also similar to the results of a randomized, double-blind, placebo-controlled study conducted in 2023 (Na Takuathung et al. 2024), which investigated the effects of the administration of a soy extract on lipid profile, blood glucose and insulin levels, on a group of 100 participants, 50 of whom received soy extract for 12 weeks, and the other 50, received a placebo. The results of this clinical trial revealed that the administration of soy extract in postmenopausal women caused a significant decrease in total cholesterol levels, compared to the group that received a placebo. Also, Takuathung et al. concluded that blood glucose values did not record a statistically significant decrease after the administration of soy extract. In our study, the comparative analysis of blood glucose values between the control group and the group that received fermented soy extract did not reveal statistically significant differences. However, glycemic values were improved in the SLM group, in which dietary supplements based on fermented soy extract were administered, but associated with lyophilized royal jelly capsules.

The number and intensity of daily hot flashes are important indicators in assessing the severity of symptoms associated with menopause. Comparing the results obtained at the second study visit in the groups treated with dietary supplements with those in the control group, we observed that both the number and intensity of hot flashes were significantly reduced after 8 weeks of treatment. The comparison between the number and intensity of hot flashes after the treatment in the groups who received dietary supplements, recorded statistically significant differences only in the number of hot flashes, thus being able to conclude that the association between dietary supplements based on fermented soyb extract and royal jelly had a superior effect in reducing the number of hot flashes compared to the solitary administration of fermented soy extract.

Isoflavones from soybeans are biologically inactive glycosides such as genistin and daidzin. After ingestion, these molecules are hydrolyzed in the gut by intestinal bacterial β-glucosidases, resulting in the formation of the corresponding bioactive aglycones: genistein and daidzein. Daidzein can be metabolized to dihydrodaidzein and then to S-equol (Balan et al. 2024). Daidzein has a proven higher bioavailability than genistein because it has a longer half-life in the gut. Therefore, the fermentation process of soybeans increases the content of more bioavailable aglycones (Pabich et al. 2019). In fermented soy products or food supplements, the values of aglycones can vary from 40 to 100% (Do Prado et al. 2022). Therefore, according to these data, the use of food supplements based on fermented soyb extract, similar to those administered to the patients in the present study, seem to have a greater efficacy against symptoms associated with menopause, especially vasomotor symptoms, compared to non-fermented soy extract. A 2019 meta-analysis by Daily et al. (Daily et al. 2019), which included 5 randomized clinical trials, with a total of 728 cases, obtained some results consistent with the results obtained in the present study. The authors concluded that the administration of equol, the product of daidzein metabolism, obtained after the fermentation process, significantly decreased the incidence and severity of hot flashes in menopausal women.

The side effects observed in the two groups treated with dietary supplements were reduced and isolated. In the S group, only one patient reported an increase of the daily hot flashes during the treatment, and two patients complained of headache, while in the SLM group only one patient complained of transient skin rash. The major royal jelly allergens are the major proteins type 1 and 2, which are similar to bee venom allergens. Royal jelly major proteins 1 and 2 can cause several allergic reactions such as asthma, dermatitis, rashes, eczema, bronchospasm, anaphylaxis, hemorrhagic colitis or even anaphylactic shock and death in some situations (Balan et al. 2020). Also, the oral allergy syndrome secondary to the consumption of royal jelly has been mentioned in the literature (Fantini et al. 2014).

CHAPTER XI. CONCLUSIONS

1. In accordance with the literature data, approximately 65-70% of the patients who consulted the specialist during the recruitment period for menopause-associated symptoms were from urban areas, graduated higher education or have higher education degrees.
2. 82.5% of the menopausal patients included in the study reported that they do not perform sports at all.
3. 49.2% of the menopausal patients included in the study reported that they do not have monthly sexual contacts.
4. In the group treated for 8 weeks with dietary supplements based on fermented soy extract, the DASS-21R score and MENQOL score improved significantly, the number and intensity of hot flashes decreased, and an increase in the spontaneous wake-up time in the morning was observed.
5. In the group treated with dietary supplements based on fermented soy extract, significant improvements were recorded in serum glucose levels, LDL cholesterol, HDL cholesterol, total cholesterol, triglycerides, salivary cortisol (collected at 12 am), and systolic blood pressure (SBP) after 8 weeks of treatment.
6. In the group treated for 8 weeks with dietary supplements based on fermented soy extract and royal jelly, the DASS-21R score and MENQOL score improved significantly, the number and intensity of hot flashes decreased, the spontaneous wake-up time in the morning increased, and the number of monthly sexual contacts increased.
7. In the group treated with dietary supplements based on fermented soy extract and royal jelly, significant improvements were recorded in serum glucose levels, LDL cholesterol, HDL cholesterol, total cholesterol, triglycerides, salivary cortisol (collected at 12 pm), diastolic blood pressure (DBP), and systolic blood pressure (SBP) after 8 weeks of administration.
8. The number of urinary tract infections significantly decreased in the SLM group after 8 weeks of administration of the dietary supplement based on fermented soy extract and lyophilized royal jelly.
9. Quality of life scores were significantly improved in the SLM group after 8 weeks of treatment with a dietary supplement based on fermented soy extract and royal jelly, compared to the S group and the control group.
10. The values of HDL cholesterol, LDL cholesterol, total cholesterol, triglycerides, and salivary cortisol were better in the SLM group compared to those recorded in the S group after 8 weeks of administration of the dietary supplement based on fermented soy extract and royal jelly.
11. Compared to the patients in the control group, those in the S group achieved a better MENQOL score (vasomotor domain), reported a decrease in the number and intensity of hot flashes, and showed improved values of LDL cholesterol, total cholesterol, triglycerides, and salivary cortisol (at 12 pm).
12. Compared to the patients in the control group, those in the SLM group achieved a better MENQOL score (with differences in all 4 subdomains), reported a decrease in the number and intensity of hot flashes, an increase in wake-up time, and improved values of glucose, LDL cholesterol, total

cholesterol, triglycerides, salivary cortisol (at 12 pm), systolic blood pressure (SBP), and diastolic blood pressure (DBP).

13. The number of urinary infections was lower in patients who received royal jelly compared to the control group.
14. 92.5% of the patients in the S group did not report any adverse effects. 97.5% of the patients in the SLM group did not report any adverse effects.
15. The MENQOL score was significantly correlated with BMI value, physical activity (only in the SLM group), daily number of hot flashes, wake-up time, and number of monthly sexual contacts.
16. The DASS-21R score was significantly correlated with the daily number of hot flashes and the number of monthly sexual contacts.
17. Patients with higher nocturnal salivary cortisol levels (at 12 pm) experienced a higher number of hot flashes, with greater intensity, and reported very early awakenings.
18. The higher the number and intensity of hot flashes, the earlier the patients' spontaneous awakenings were.

CHAPTER XIII. ORIGINALITY AND NOVEL CONTRIBUTIONS OF THE WORK. FUTURE RESEARCH DIRECTIONS. DISSEMINATION OF RESULTS

The results of this observational, comparative follow-up study on the evolution of patients in menopause or menopausal transition treated with two types of non-hormonal dietary supplements under usual medical practice conditions significantly contribute to the limited body of data currently available in the specialized literature. Although they do not allow conclusions about cause-effect relationships, prospective observational studies are a valid method of investigating treatment evolution, reflecting everyday medical practice, and providing new data on less investigated aspects. Despite not being a randomized study, the comparative analysis of the characteristics of the patients in the three samples at the time of inclusion was not statistically significant, suggesting a certain homogeneity of the studied population and a reduced risk of bias.

This doctoral thesis is based on an extensive study involving the statistical analysis of a large number of variables, with the main objective of comparing the effects observed following the administration of two different treatment schemes, including phytotherapeutic compounds. The analysis of the study's results, which can be considered a reference point for specialists in the field who wish to recommend non-hormonal natural compounds for the management of menopause-related symptoms, along with the review of bibliographic materials, has allowed for the formulation of conclusions with applicability in medical practice, without referring to cause-effect relationships.

In this context, the present study is the first in the specialized literature to analyze the effects of consuming lyophilized royal jelly in combination with a compound based on fermented soy extract on vasomotor symptoms, sleep disorders, and sexual disorders associated with menopause, as well as on a wide range of clinical and paraclinical parameters.

The particular epidemiological context imposed by the COVID-19 pandemic, during which this study was conducted, represents another aspect of originality. The fear of illness and the particular operating conditions of hospitals during this period considerably reduced the already low accessibility of

menopausal patients to specialists. In this context, the compliance rate of patients with a natural treatment, without synthetic or hormonal components, nevertheless increased.

In conclusion, this study offers an innovative perspective on the administration of natural, non-hormonal dietary supplements for managing menopause symptoms, while also highlighting the necessity and importance of a multidisciplinary approach in improving the quality of life for patients.

Future research directions

- Researching the positive and negative effects of other natural dietary supplements on menopause-related symptoms, as well as their cumulative effects, with the aim of optimizing the management of these symptoms and improving the quality of life for affected women.
- Comparing the beneficial effects exerted by natural compounds with the effects generated by hormone replacement therapy.
- Developing and implementing local and/or national programs to inform patients about the possibility of treating menopause-associated symptoms with natural compounds, without synthetic hormonal components.
- Increasing accessibility to menopause specialists and eliminating the "doomed patient" status for women who have reached menopause.

Dissemination of the research results in the academic environment.

Both the results of the personal research within the doctoral thesis and those based on related topics have been published in WOS Clarivate-indexed journals and BDI-indexed journals, both national and international. From the doctoral thesis topic, 4 articles have been published in WOS Clarivate-indexed journals, 2 articles in BDI-indexed journals, and 1 article in a Proceedings ISI-indexed journal. Additionally, the results from the thesis content and related topics have been presented as papers at national and international conferences and congresses, with 2 of these being published in ISI Proceedings volumes (10 papers).

REFERENCES

Agarwal, S., Alzahrani, F. A. and Ahmed, A. (2018). Hormone Replacement Therapy: Would it be Possible to Replicate a Functional Ovary? *Int J Mol Sci*, 19(10): 3160.DOI: 10.3390/ijms19103160.

Al-Safi, Z. A. and Santoro, N. (2014). Menopausal hormone therapy and menopausal symptoms. *Fertil Steril*, 101(4): 905-915.DOI: 10.1016/j.fertnstert.2014.02.032.

Ambikairajah, A., Walsh, E. and Cherbuin, N. (2019). Lipid profile differences during menopause: a review with meta-analysis. *Menopause*, 26(11): 1327-1333.DOI: 10.1097/gme.0000000000001403.

Ambikairajah, A., Walsh, E., Tabatabaei-Jafari, H. and Cherbuin, N. (2019). Fat mass changes during menopause: a metaanalysis. *Am J Obstet Gynecol*, 221(5): 393-409.e350.DOI: 10.1016/j.ajog.2019.04.023.

Balan, A., Moga, M. A., Dima, L., Dinu, C. G., Martinescu, C. C., Panait, D. E., et al. (2021). An Overview on the Conservative Management of Endometriosis from a Naturopathic Perspective: Phytochemicals and Medicinal Plants. *Plants (Basel)*, 10(3).DOI: 10.3390/plants10030587.

Balan, A., Moga, M. A., Dima, L., Toma, S., Elena Neculau, A. and Anastasiu, C. V. (2020). Royal Jelly-A Traditional and Natural Remedy for Postmenopausal Symptoms and Aging-Related Pathologies. *Molecules*, 25(14).DOI: 10.3390/molecules25143291.

Balan, A., Moga, M. A., Neculau, A. E., Mitrica, M., Rogozea, L., Ifteni, P., et al. (2024). Royal Jelly and Fermented Soy Extracts—A Holistic Approach to Menopausal Symptoms That Increase the Quality of Life in Pre- and Post-menopausal Women: An Observational Study. 16(5): 649.

Bar-Shavit, Z. (2007). The osteoclast: a multinucleated, hematopoietic-origin, bone-resorbing osteoimmune cell. *J Cell Biochem*, 102(5): 1130-1139.DOI: 10.1002/jcb.21553.

Beraldo, R. A., Meliscki, G. C., Silva, B. R., Navarro, A. M., Bollela, V. R., Schmidt, A., et al. (2018). Anthropometric measures of central adiposity are highly concordant with predictors of cardiovascular disease risk in HIV patients. *Am J Clin Nutr*, 107(6): 883-893.DOI: 10.1093/ajcn/nqy049.

Birkhäuser, M. (2002). Depression, menopause and estrogens: is there a correlation? *Maturitas*, 41 S3-8.DOI: 10.1016/s0378-5122(02)00009-9.

Blümel, J. E., Chedraui, P., Baron, G., Belzares, E., Bencosme, A., Calle, A., et al. (2011). A large multinational study of vasomotor symptom prevalence, duration, and impact on quality of life in middle-aged women. *Menopause*, 18(7): 778-785.DOI: 10.1097/gme.0b013e318207851d.

Bobescu, E., **Bălan, A.**, Moga, M. A., Teodorescu, A., Mitrică, M. and Dima, L. (2020). Are There Any Beneficial Effects of Spirulina Supplementation for Metabolic Syndrome Components in Postmenopausal Women? *Mar Drugs*, 18(12).DOI: 10.3390/md18120651.

Bondarev, D., Finni, T., Aukee, P., Kokko, K., Kujalav, U., Kovanen, V., et al. (2019). Effect of the Menopausal Transition on Physical Performance: A Longitudinal Study. *Med Sci Sports Exercise*, 51(6): 572-572.DOI: 10.1249/01.mss.0000562220.91407.5f.

Booth, N. L., Piersen, C. E., Banuvar, S., Geller, S. E., Shulman, L. P. and Farnsworth, N. R. (2006). Clinical studies of red clover (Trifolium pratense) dietary supplements in menopause: a literature review. *Menopause*, 13(2): 251-264.DOI: 10.1097/01.gme.0000198297.40269.f7.

Bruce, D. and Rymer, J. (2009). Symptoms of the menopause. *Best Pract Res Clin Obstet Gynaecol*, 23(1): 25-32.DOI: 10.1016/j.bpobgyn.2008.10.002.

Burger, H. G., Dudley, E. C., Robertson, D. M. and Dennerstein, L. (2002). Hormonal changes in the menopause transition. *Recent Prog Horm Res*, 57: 257-275.DOI: 10.1210/rp.57.1.257.

Carboni, L., McCarthy, D. J., Delafont, B., Filosi, M., Ivanchenko, E., Ratti, E., et al. (2019). Biomarkers for response in major depression: comparing paroxetine and venlafaxine from two randomised placebo-controlled clinical studies. *Translat Psychiatry*, 9(1): 182.DOI: 10.1038/s41398-019-0521-7.

Carley, M. E., Rickard, D. J., Gebhart, J. B., Webb, M. J., Podratz, K. C. and Spelsberg, T. C. (2003). Distribution of estrogen receptors alpha and beta mRNA in mouse urogenital tissues and their expression after oophorectomy and estrogen replacement. *Int Urogynecol J Pelvic Floor Dysfunct*, 14(2): 141-145.DOI: 10.1007/s00192-002-1020-5.

Carr, M. C., Kim, K. H., Zambon, A., Mitchell, E. S., Woods, N. F., Casazza, C. P., et al. (2000). Changes in LDL density across the menopausal transition. *J Investig Med*, 48(4): 245-250.

Conneely, O., Mulac-Jericevic, B., Demayo, F., Lydon, J. and O'Malley, B. (2002). Reproductive functions of progesterone receptors. *Recent Progress Hormone Res*, 57: 339-355.DOI: 10.1177/1071557600007001S09.

Conneely, O. M. and Lydon, J. P. (2000). Progesterone receptors in reproduction: functional impact of the A and B isoforms. *Steroids*, 65(10-11): 571-577.DOI: 10.1016/s0039-128x(00)00115-x.

Coriano, C. G., Liu, F., Sievers, C. K., Liang, M., Wang, Y., Lim, Y., et al. (2018). A Computational-Based Approach to Identify Estrogen Receptor Heterodimer Selective Ligands. *Mol Pharmacol*, 93(3): 197-207.DOI: 10.1124/mol.117.108696

Daily, J. W., Ko, B.-S., Ryuk, J., Liu, M., Zhang, W. and Park, S. (2019). Equol Decreases Hot Flashes in Postmenopausal Women: A Systematic Review and Meta-Analysis of Randomized Clinical Trials. *J Med Food*, 22(2): 127-139.DOI: 10.1089/jmf.2018.4265.

Do Prado, F. G., Pagnoncelli, M. G. B., de Melo Pereira, G. V., Karp, S. G. and Soccol, C. R. (2022). Fermented soy products and their potential health benefits: A review. *Microorganisms*, 10(8): 1606.DOI: 10.3390/microorganisms10081606.

Edelman, A., Gallo, M. M., Jensen, J., Nichols, M., Schulz, K. and Grimes, D. A. (2004). Continuous or extended cycle vs. cyclic use of combined oral contraceptives for contraception. *Cochrane Database of Systematic Reviews*, (1).DOI: 10.1002/14651858.CD004695.

Fantini, P., Delle Donne, P., Calogiuri, G., Ferrannini, A., Vacca, A., Nettis, Eustachio and Di Leo, E. (2014). Oral Allergy Syndrome in a Child Provoked by Royal Jelly. *Case Rep Med*, 2014(1): 941248.DOI: 10.1155/2014/941248.

Ferrara, C. M., Lynch, N. A., Nicklas, B. J., Ryan, A. S. and Berman, D. M. (2002). Differences in adipose tissue metabolism between postmenopausal and perimenopausal women. *J Clin Endocrinol Metab*, 87(9): 4166-4170.DOI: 10.1210/jc.2001-012034.

Ford, E. S., Giles, W. H. and Dietz, W. H. (2002). Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. *JAMA*, 287(3): 356-359.DOI: 10.1001/jama.287.3.356.

Gencturk, N., Bilgic, F. Ş. and Kaban, H. U. (2024). The effect of soy isoflavones given to women in the climacteric period on menopausal symptoms and quality of life: Systematic review and meta-analysis of randomized controlled trials. *Explore*.DOI: 10.1016/j.explore.2024.05.010.

Giangrande, P. H., Kimbrel, E. A., Edwards, D. P. and McDonnell, D. P. (2000). The opposing transcriptional activities of the two isoforms of the human progesterone receptor are due to differential cofactor binding. *Mol Cell Biol*, 20(9): 3102-3115.DOI: 10.1128/mcb.20.9.3102-3115.2000.

Gregersen, I., Høibraaten, E., Holven, K. B., Løvdahl, L., Ueland, T., Mowinckel, M.-C., et al. (2019). Effect of hormone replacement therapy on atherogenic lipid profile in postmenopausal women. *Thrombosis Res*, 184: 1-7.DOI: 10.1016/j.thromres.2019.10.005.

Gustafsson, J. A. (2003). What pharmacologists can learn from recent advances in estrogen signalling. *Trends Pharmacol Sci*, 24(9): 479-485.DOI: 10.1016/s0165-6147(03)00229-3.

Harlow, S. D., Gass, M., Hall, J. E., Lobo, R., Maki, P., Rebar, R. W., et al. (2012). Executive summary of the Stages of Reproductive Aging Workshop + 10: addressing the unfinished agenda of staging reproductive aging. *J Clin Endocrinol Metab*, 97(4): 1159-1168.DOI: 10.1210/jc.2011-3362.

Heldring, N., Pike, A., Andersson, S., Matthews, J., Cheng, G., Hartman, J., et al. (2007). Estrogen receptors: how do they signal and what are their targets. *Physiol Rev*, 87(3): 905-931.DOI: 10.1152/physrev.00026.2006.

Hill, D. A., Crider, M. and Hill, S. R. (2016). Hormone Therapy and Other Treatments for Symptoms of Menopause. *Am Fam Physician*, 94(11): 884-889.

Imhof, M., Gocan, A., Imhof, M. and Schmidt, M. (2018). Soy germ extract alleviates menopausal hot flushes: placebo-controlled double-blind trial. *Eur J Clin Nutr*, 72(7): 961-970.DOI: 10.1038/s41430-018-0173-3.

Jehan, S., Masters-Isarilov, A., Salifu, I., Zizi, F., Jean-Louis, G., Pandi-Perumal, S. R., et al. (2015). Sleep Disorders in Postmenopausal Women. *Journal Sleep Dis Ther*, 4(5): 1000212.DOI: 10.4172/2167-0277.1000212.

Jensen, E. V. (1962). On the mechanism of estrogen action. *Perspect Biol Med*, 6: 47-59.DOI: 10.1353/pbm.1963.0005.

Johnson, A., Roberts, L. and Elkins, G. (2019). Complementary and Alternative Medicine for Menopause. *J Evidence Based Integrat Med*, 24: 2515690X19829380.DOI: 10.1177/2515690x19829380.

Katzenellenbogen, B. S., Montano, M. M., Ediger, T. R., Sun, J., Ekena, K., Lazennec, G., et al. (2000). Estrogen receptors: selective ligands, partners, and distinctive pharmacology. *Recent Prog Horm Res*, 55: 163-193; discussion 194-165.

Khan, J. A., Amazit, L., Bellance, C., Guiochon-Mantel, A., Lombès, M. and Loosfelt, H. (2011). p38 and p42/44 MAPKs differentially regulate progesterone receptor A and B isoform stabilization. *Mol Endocrinol*, 25(10): 1710-1724.DOI: 10.1210/me.2011-1042.

Kostenuik, P. J. (2005). Osteoprotegerin and RANKL regulate bone resorption, density, geometry and strength. *Curr Opin Pharmacol*, 5(6): 618-625.DOI: 10.1016/j.coph.2005.06.005.

Krause, M. S. and Nakajima, S. T. (2015). Hormonal and nonhormonal treatment of vasomotor symptoms. *Obstet Gynecol Clin North Am*, 42(1): 163-179.DOI: 10.1016/j.ogc.2014.09.008.

Kuiper, G. G., Enmark, E., Pelto-Huikko, M., Nilsson, S. and Gustafsson, J. A. (1996). Cloning of a novel receptor expressed in rat prostate and ovary. *Proceedings of the National Academy of Sciences of the United States of America*, 93(12): 5925-5930.DOI: 10.1073/pnas.93.12.5925.

Lavery, D. N. and McEwan, I. J. (2005). Structure and function of steroid receptor AF1 transactivation domains: induction of active conformations. *Biochem J*, 391(Pt 3): 449-464.DOI: 10.1042/bj20050872.

Leygue, E., Dotzlaw, H., Lu, B., Glor, C., Watson, P. H. and Murphy, L. C. (1998). Estrogen receptor beta: mine is longer than yours? *J Clin Endocrinol Metab*, 83(10): 3754-3755.DOI: 10.1210/jcem.83.10.5187-1.

Liu, M. M., Albanese, C., Anderson, C. M., Hilty, K., Webb, P., Uht, R. M., et al. (2002). Opposing action of estrogen receptors alpha and beta on cyclin D1 gene expression. *J Biol Chem*, 277(27): 24353-24360.DOI: 10.1074/jbc.M201829200.

Lopes, C. M. C., Lima, S. M. R. R., Veiga, E. C. d. A., Soares-Jr, J. M. and Baracat, E. C. (2019). Phytotherapeutic medicines: reality or myth? *Revista da Associação Médica Brasileira*, 65: 292-294.

Lu, B., Leygue, E., Dotzlaw, H., Murphy, L. J. and Murphy, L. C. (2000). Functional characteristics of a novel murine estrogen receptor-beta isoform, estrogen receptor-beta 2. *J Mol Endocrinol*, 25(2): 229-242.DOI: 10.1677/jme.0.0250229.

Mahady, G. B., Fabricant, D., Chadwick, L. R. and Dietz, B. (2002). Black Cohosh: An Alternative Therapy for Menopause? *Nutr Clin Care*, 5(6): 283-289.DOI: 10.1046/j.1523-5408.2002.05603.x.

Martinkovich, S., Shah, D., Planey, S. L. and Arnott, J. A. (2014). Selective estrogen receptor modulators: tissue specificity and clinical utility. *Clin Interv Aging*, 9: 1437-1452.DOI: 10.2147/cia.S66690.

Matthews, K. A., Meilahn, E., Kuller, L. H., Kelsey, S. F., Caggiula, A. W. and Wing, R. R. (1989). Menopause and risk factors for coronary heart disease. *N Engl J Med*, 321(10): 641-646.DOI: 10.1056/nejm198909073211004.

Mauriège, P., Imbeault, P., Prud'Homme, D., Tremblay, A., Nadeau, A. and Després, J. P. (2000). Subcutaneous adipose tissue metabolism at menopause: importance of body fatness and regional fat distribution. *J Clin Endocrinol Metab*, 85(7): 2446-2454.DOI: 10.1210/jcem.85.7.6687.

Moga, M. A., Dimienescu, O. G., Bălan, A., Dima, L., Toma, S. I., Bîgiu, N. F., et al. (2021). Pharmacological and Therapeutic Properties of Punica granatum Phytochemicals: Possible Roles in Breast Cancer. *Molecules* 26(4): 1054.DOI: 10.3390/molecules26041054.

Na Takuathung, M., Teekachunhatean, S., Chansakaow, S., Klinjan, P., Inpan, R., Kongta, N., et al. (2024). The effects of SOY extract nutraceuticals on postmenopausal women's health: A randomized, double-blind, placebo-controlled trial. *Journal of Functional Foods*, 113: 106055.DOI: 10.1016/j.jff.2024.106055.

Pabich, M. and Materska, M. (2019). Biological Effect of Soy Isoflavones in the Prevention of Civilization Diseases. 11(7): 1660.

Palacios, S. and Cancelo, M. J. (2016). Clinical update on the use of ospemifene in the treatment of severe symptomatic vulvar and vaginal atrophy. *Int J Womens Health*, 8: 617-626.DOI: 10.2147/ijwh.S110035.

Panay, N., Briggs, P. and Kovacs, G. (2015). The history and politics of menopause

Managing the menopause: 21st century solutions. Cambridge University Press.DOI: 10.1017/CBO9781316091821.

Polo-Kantola, P., Saaresranta, T. and Polo, O. (2001). Aetiology and Treatment of Sleep Disturbances During Perimenopause and Postmenopause. *CNS Drugs*, 15(6): 445-452.DOI: 10.2165/00023210-200115060-00003.

Pouliot, M. C., Després, J. P., Nadeau, A., Moorjani, S., Prud'Homme, D., Lupien, P. J., et al. (1992). Visceral obesity in men. Associations with glucose tolerance, plasma insulin, and lipoprotein levels. *Diabetes*, 41(7): 826-834.DOI: 10.2337/diab.41.7.826.

Prior, J. C. and Hitchcock, C. L. (2011). The endocrinology of perimenopause: need for a paradigm shift. *Front Biosci* 3: 474-486.DOI: 10.2741/s166.

Ruiz, A. D. and Daniels, K. R. (2014). The effectiveness of sublingual and topical compounded bioidentical hormone replacement therapy in postmenopausal women: an observational cohort study. *Int J Pharm Compd*, 18(1): 70-77.

Sharif, S. N. and Darsareh, F. (2019). Effect of royal jelly on menopausal symptoms: A randomized placebo-controlled clinical trial. *Complement Ther Clin Practice*, 37: 47-50.DOI: 10.1016/j.ctcp.2019.08.006.

Soltysik, K. and Czekaj, P. (2013). Membrane estrogen receptors - is it an alternative way of estrogen action? *J Physiol Pharmacol*, 64(2): 129-142.

Spiegelman, B. M. and Flier, J. S. (2001). Obesity and the regulation of energy balance. *Cell*, 104(4): 531-543.DOI: 10.1016/s0092-8674(01)00240-9.

Tadir, Y., Gaspar, A., Lev-Sagie, A., Alexiades, M., Alinsod, R., Bader, A., et al. (2017). Light and energy based therapeutics for genitourinary syndrome of menopause: Consensus and controversies. *Lasers Surg Med*, 49(2): 137-159.DOI: 10.1002/lsm.22637.

Villella, S. J. A. J. o. H. M. (2016). The clinical management of menstrual migraine and headache by the herbal medicine practitioner. *Australian J Herb Med.* , 28(3): 75.

Wang, Z., Zhang, X., Shen, P., Loggie, B. W., Chang, Y. and Deuel, T. F. (2005). Identification, cloning, and expression of human estrogen receptor-alpha36, a novel variant of human estrogen receptor-alpha66. *Biochem Biophy Res Comm*, 336(4): 1023-1027.DOI: 10.1016/j.bbrc.2005.08.226.

Weinberg, M. E., Manson, J. E., Buring, J. E., Cook, N. R., Seely, E. W., Ridker, P. M., et al. (2006). Low sex hormone–binding globulin is associated with the metabolic syndrome in postmenopausal women. *Metabolism*, 55(11): 1473-1480.DOI: 10.1016/j.metabol.2006.06.017.

Yang, S., Zeng, Q., Huang, X., Liang, Z. and Hu, H. (2023). Effect of Isoflavones on Blood Lipid Alterations in Postmenopausal Females: A Systematic Review and Meta-Analysis of Randomized Trials. *Advances in Nutrition*, 14(6): 1633-1643.DOI: 10.1016/j.advnut.2023.09.008.

Zhou, C., Wu, Q., Wang, Z., Wang, Q., Liang, Y. and Liu, S. (2020). The Effect of Hormone Replacement Therapy on Cognitive Function in Female Patients With Alzheimer's Disease: A Meta-Analysis. *Am J Alzheimer Dis Other Dementias*, 35: 1533317520938585.DOI: 10.1177/1533317520938585.

LIST OF PUBLICATIONS ACHIEVED THROUGH THE DOCTORAL STUDIES PROGRAM

ARTICLES RELATED TO THE DOCTORAL THESIS

1. **Bălan, A.,** Moga, M.A, Neculau, A.E., Mitrica, M., Rogozea, L., Ifteni P., Dima L. Royal Jelly and Fermented Soy Extracts—A Holistic Approach to Menopausal Symptoms That Increase the Quality of Life in Pre- and Post-menopausal Women: An Observational Study. Nutrients 2024, 16(5), 649. Disponibil online https://doi.org/10.3390/nu16050649 - WOS Clarivate indexed journal, IF (2023) 4.8.
2. **Bălan A.**, Moga M.A., Dima L., Toma S., Neculau A.E., Anastasiu C.V. Royal Jelly-A Traditional and Natural Remedy for Postmenopausal Symptoms and Aging-Related Pathologies. Molecules. 2020, 25(14): 3291. Disponibil online https://pubmed.ncbi.nlm.nih.gov/32698461/- WOS Clarivate indexed journal, IF (2020) 4.4.
3. Bobescu E., **Bălan A.***, Moga M.A., Teodorescu A., Mitrica M., Dima L. Are There Any Beneficial Effects of Spirulina Supplementation for Metabolic Syndrome Components in Postmenopausal Women? Marine Drugs. 2020, 18(12), 651. Disponibil online https://www.mdpi.com/1660-3397/18/12/651- WOS Clarivate indexed journal, IF (2020) 5.11
4. Dima, L.; **Bălan, A.***; Moga, M.A.; Dinu, C.G.; Dimienescu, O.G.; Varga, I.; Neculau, A.E. Botulinum Toxin a Valuable Prophylactic Agent for Migraines and a Possible Future Option for the Prevention of Hormonal Variations-Triggered Migraines. Toxins, 2019, 11. Disponibil online https://www.mdpi.com/2072-6651/11/8/465. WOS Clarivate indexed journal, IF (2019) 3.5
5. Moga, M.A., Dimienescu, O.G., Bigiu, N.F., **Bălan, A.**, Ples, L. Postmenopausal vulvovaginal atrophy - a systematic review of a public health problem solved with CO2 Laser therapy. Archives of the Balkan Medical Union. 2018, Suppl 1, 53.
6. **Bălan, A.**, Dima, L., Varga, I., Bîgiu, N.F., Moga, S. Management strategies of the symptomatology and pathology associated with menopause- an overview. Bulletin of the Transilvania University of Braşov Series VI: Medical Sciences ◘ Vol. 12 (61) No. 2 – 2019. Disponibil online https://www.researchgate.net/publication/339767166_Management_Strategies_of_The_Symptomatology_and_Pathology_Associated_with_Menopause_-_An_Overview
7. **Bălan A.,** Dvornic P, Nisioi C, Martinescu C., Panait D., Moga M.A. Hormonal fluctuations related to depressive symptoms in menopause. Bulletin of the Transilvania University of Braşov Series VI: Medical Sciences ◘ Vol. 13 No. 2 – 2020. Disponibil online http://webbut.unitbv.ro/index.php/Series_VI/article/view/533/470

ARTICLES PUBLISHED IN WOS CLARIVATE INDEXED JOURNALS

1. Moga M.A., Bălan A., Anastasiu CV, Dimienescu OG, Neculoiu CD, Gavriș C. An Overview on the Anticancer Activity of Azadirachta indica (Neem) in Gynecological Cancers. Int J Mol Sci. 2018, 19(12). https://pubmed.ncbi.nlm.nih.gov/30563141/ - IF (2018) 4.18
2. Moga, MA, Bălan A, Dimienescu OG, Burtea V, Dragomir MR, Anastasiu CV. Circulating miRNAs as Biomarkers for Endometriosis and Endometriosis-Related Ovarian Cancer— An Overview. JCM. 2019, 8(5). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6571871/ IF (2019) 5.68
3. Moga M.A., Dima L., Bălan A*, Blidaru A., Dimienescu O.G., Podasca C., Toma S. Are Bioactive Molecules from Seaweeds a Novel and Challenging Option for the Prevention of HPV Infection and Cervical Cancer Therapy? - A Review. Int J Mol Sci. 2021, 22(2), 629. Disponibil online https://www.mdpi.com/1422-0067/22/2/629 IF (2021) 6.20 – corresponding author
4. Bălan A., Moga M.A., Dima L., Dinu C.G., Martinescu C.C., Panait D.E., Irimie C.A., Anastasiu C.V. An Overview on the Conservative Management of Endometriosis from a Naturopathic Perspective: Phytochemicals and Medicinal Plants. Plants. 2021, 10, 587. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8003677/ IF (2021) 4.65
5. Moga M.A., Dimienescu O.G., Bălan A, Dima L., Toma S.I., Bigiu N.F, Blidaru A. Pharmacological and Therapeutic Properties of Punica granatum Phytochemicals: Possible Roles in Breast Cancer. Molecules, 2021, 26(4), 1052. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7921999/ IF (2021) 4.92
6. Bobescu, E., Marceanu, L.G., Dima, L, Bălan, A, Strempel, C.G., Covaciu, A. Trimetazinide Therapy in Coronary Artery Disease: The Impact on Oxidative Stress, Inflammation, Endothelial Dysfunction, and Log-Term Prognosis. Am J Ther. 2021, 28(5). Disponibil online https://pubmed.ncbi.nlm.nih.gov/34321406/ IF (2021) 3.09

ARTICLES PUBLISHED IN JOURNALS INDEXED PROCEEDINGS ISI

1. Moga, M. A., **Bălan, A.,** Liana, P., Dimienescu, O., Vasile, I., Anastasiu, C., Ungureanu, D. (2019). EP19. 25: A prospective study of Doppler velocimetry indices and the accurate management in pregnancies with intrauterine growth-restricted fetuses. Proceedings ISI :Ultrasound in Obstetrics & Gynecology, 54, 358-359.
2. Dimienescu, O., **Bălan, A.**, Liana, P., Moga, S., Podasca, C., Bigiu, N., & Panait, D. (2019). EP07. 02: An overview of the cerebral abnormalities ultrasonographically detected at fetuses with congenital cytomegalovirus infection. Proceedings ISI :Ultrasound in Obstetrics & Gynecology, 54, 268-269.

ARTICLES PUBLISHED IN BDI INDEXED JOURNALS

1. Moga, M.A., Podașcă, C., Varga, I., Dimienescu, O.G., Anastasiu, C.V., Aldea, F., **Bălan, A**. Congenital cytomegalovirus infection in pregnancy- an overview of the fetal abnormalities detected by ultrasound. Bulletin of the Transilvania University of Braşov Series VI: Medical Sciences Vol. 11 (60) No. 2 – 2018. https://webbut.unitbv.ro/index.php/Series_VI/article/view/1288 .
2. Moga MA, **Bălan A**, Liana P, Dimienescu OG, Vasile I, Anastasiu CV, Ungureanu. A prospective study of Doppler velocimetry indices and the accurate management in pregnancies with intrauterine growth-restricted fetuses. 29th World Congress on Ultrasound in Obstetrics and Gynecology. 2019; 54 (Suppl. 1): 358.
3. **Bălan A**, Moga M, Dimienescu OG, Arvatescu C, Dima L. The Value Of High Resolution Chromosomal Microarray In Fetuses With Normal Karyotype And Increased Nuchal Translucency. J Perinat. Med. 2019, 47(1). Doi: 10.1515/jpm-2019-2501.
4. Dimienescu OG, Moga M, Arvatescu C, **Bălan A**, Aldea F, Anastasiu CV. Preterm birth –risk factors and predictors in a tertiary hospital in Romania. J Perinat. Med. 2019, 47, eA382. Doi: 10.1515/jpm-2019-2501.
5. Dimienescu, O.G., Moga, M.A., Aldea, F, Anastasiu, C.V., Arvatescu, C.A., **Bălan, A**. Diagnostic şi prognostic ecografic în infecțiile cu CMV din sarcină – review. Giecologia.ro, 2019, 23(1), ISSN 2457-5666.

ARTICLES PUBLISHED IN VOLUMES OF NATIONAL AND INTERNATIONAL CONFERENCES

1. Anastasiu CV, **Bălan A**, Dimienescu OG, Arvatescu CA, Moga MA. Idiopathic peripheral facial palsy in pregnancy: a predictor for preeclampsia. a case report and literature review. The XXVIth European Congess of Perinatal Medicine, St. Petersburg, Rusia, 5-8 Sept 2018.
2. **Bălan A**.; Anastasiu C.V.; Arvătescu C.A.; Bîgiu N.F; Dimienescu O.G:; Moga M.A. The efficiency of bromcriptine as a prolactin antagonist in treatment of peripartum cardiomiopathy – a sistematic review. Al 17 lea Congres al Societății de Obstetrică și Ginecologie din Romania, 20-22 septembrie 2018, Iasi
3. Dimienescu O.G., Moga M.A., Aldea F., Anastasiu C.V, **Bălan A**. Ultrasonographic Diagnosis and Long-term Prognosis of Cytomegalovirus Infection In Pregnancy – A systematic review. Al 7-lea Congres al Societății de Obstetrică și Ginecologie din România, 11-13 aprilie 2019, Targu Mures.
4. Dimienescu, O.G., Moga, M.A., Arvătescu, C.A**., Bălan, A**., Aldea, F., Anastasiu, C.V. Preterm birth – risk factors and predictors in a tertiary hospital in Romania. World Congress of Perinatal Medicine, 11-14 September 2019, Istanbul.

5. Ungureanu, D., **Bălan, A.**, Suciu, L.M., Moga, M.A., Podașcă, C., Anastasiu, C.V. Outcomes of preterm infants born ar 25-32 weeks of gestation according to the place of birth and perinatal interventions. World Congress of Perinatal Medicine, 11-14 September 2019, Istanbul.
6. Moga, M.A., **Bălan, A.**, Dimienescu, O.G., Sima, R., Marceanu, L.G., Dima, L. 4. Seaweeds - The Aquatic Miracle in the Fight Against Human Papilloma Virus and Cervical Cancer. The 5th International Conference – New Trends on Sensing-Monitoring-Telediagnosis for Life Sciences, 3-4 July 2021, Bucharest, Romania.
7. Moga, M.A., Dimienescu, O.G., **Bălan, A.,** Aldea, F., Dima, L., Sima, R. 5. Marine algae – novel candidates for the prevention and treatment of HPV infection and cervical cancer. The 3rd edition of the summer school - Food Safety And Healthy Living. 5-6 July, Brasov, Romania.
8. **Bălan A.**, Badea M., Moga M.A., Anastasiu C.V., Gavris C., Dima L. Suplimentarea dietei cu Spirulină la femeile postmenopauzale: efecte potențial benefice ale acesteia împotriva componentelor sindromului metabolic. În Book of Abstracts - Romanian Ethnopharmacology on its 20 th Anniversary Simposium (coordinator: Prof.dr. Angela Marculescu), Sirnea, judetul Brasov, 17-20 iunie, 2021; pag. 63; ISSN: 1844 – 6604.

BOOK CHAPTERS

1. Moga M.A., **Bălan A.**, Dimienescu OG, Dima L. Complementary/ alternative treatment in breast cancer: complement or crime? Ο ΓΚΟ ΛΟΓΙΚΟΙ & ΕΝΔΟΣΚΟΠΙΚΟΙ ΠΡΟΒΛΗΜΑΤΙΣΜΟΙ (2019). ISBN 978-960-599-255-2.
2. Moga, M.A., Aldea, F., **Bălan, A.**, Dimienescu, O.G., Cobelschi, C., Panait, D.E. The association between the Bisphenol A and polycystic ovary syndrome. In Food Safety Monitoring and Healthy Living. 2019. Editura Universității Transilvania din Brașov. ISBN 978-606-19-1140-0.