Effectiveness and safety of using a succinate-based dietary supplement for women in menopause

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Objective of this study was to evaluate the effectiveness and safety of using a succinate-based dietary supplement complex (SBDSC) to relieve vasomotor and psychosomatic symptoms in perimenopausal and postmenopausal women.

Methods. Randomized, double-blind, placebo-controlled trial was conducted among women 42—60 years of age with vasomotor and psychosomatic menopausal symptoms. The study included medical evaluations, questionnaires using the Greene Climacteric Scale and the Spielberger-Hanin test, evaluation of plasma levels of gonadotropins, estradiol, leptin, and apolipoproteins.

Results. The study included 104 women, randomized to two groups. Analysis of the Greene Climacteric Scale results showed a statistically significant ($p<0.05$) improvement in 13 out of 21 menopausal symptoms in patients who took SBDSC. During the course of the SBDSC treatment and by the end of the study the patients showed significant changes in the levels of estradiol and gonadotropins, decrease in the average body weight and waist circumference. The Spielberger-Hanin test showed that SBDSC supplementation allows for stabilization of patients' psychological status with significant reduction in anxiety, increased resistance to stress, and improved adaptability. Comparative analysis of vital signs, blood and urine tests did not show any negative effects of SBDSC.

Conclusion: using SBDSC during menopause and postmenopause can be considered a method to relieve vasomotor and psychosomatic menopausal symptoms for women who have counter indications for HRT or do not wish to undergo HRT.

Key Words: menopause, vasomotor and psychosomatic menopausal symptoms, mitochondrial dysfunction, treatment of menopausal symptoms, ammonium succinate.

Climacteric is a physiological period in women's lives, during which changes in the reproductive system that ultimately lead to the loss of fertility. Gradual decline in the hormones secreted by the ovaries during menopausal transition is accompanied by many responses from other organs, worsened by the general aging and often leading to the formation of pathological symptoms that are generally termed "climacteric syndrome" [1, 2, 3].

The classical manifestation of the climacteric syndrome are vasomotor symptoms (hot flashes and night sweats), observed in about 80% of women in perimenopause [4, 5].

Psychosomatic symptoms are also common among women over 40. Together with hot flashes, these symptoms significantly affect the quality of life [3]. The causes of menopausal symptoms lie not only in declining production of ovarian steroids, but also in aging of the central nervous system (CNS), specifically the hypothalamus [6, 7]. Disruptions of neuronal connections, changes in the sensitivity to peripheral inputs, disruptions in the metabolism of neurohormones – all lead to the development of vasomotor and psychosomatic symptoms of climacteric syndrome and also facilitate cognitive and psychological dysfunctions [8, 9]. Progressive changes in metabolism and hormone levels during menopausal transition are the cause of changes in one's appearance. This can act as additional psychologically traumatic factors that further lower the quality of life and disrupt women's social adaptation.

The gold standard for the treatment of climacteric syndrome is rightly considered to be menopause hormone therapy (MHT) [10, 11, 12], which employs synthetic analogues of female sex steroids, mainly estrogens. Hormone therapy can be prescribed long term if a woman is regularly evaluated by a specialist. However, there are certain limitations when it comes to using this type of treatment since HRT carries known risks [11, 13, 14]. There are also contraindications for MHT and a cautious approach to it both among doctors and patients. Thus, there is an ongoing search for alternative methods of menopausal symptom relief, prophylactic treatment of age-related diseases, and improvement of life quality during perimenopause and postmenopause.

Looking at the aging reproductive system in the context of general aging, it is important to remember the close relationship between hormonal and metabolic diseases and dysfunctions. This relationship lies at the foundation of menopausal metabolic syndrome [15]. The metabolic syndrome, in turn, as a combination of different risk factors, leads to the development of atherosclerosis, acute thrombotic events, and type II diabetes. However, metabolic syndrome is a reversible condition; hence, modern diagnosis and treatment of the syndrome are the first prophylactic line of defense against the above-mentioned conditions. Hormone therapy can positively influence some aspects of the metabolic syndrome [16, 17, 18, 19, 20], but due to multiple effects of hormones on metabolic processes, the results of the treatment cannot always be predicted or be a positive one. Therefore, it is important to search for therapies that can optimize patients' metabolic status.

A number of various non-hormonal treatments, such as psychotropic medications, phytoestrogens and other herbal remedies, multivitamin complexes are used to improve women's quality of life during menopausal transition [21, 22, 23, 24]. Among these remedies, a succinate-based dietary supplement complex (SBDSC) is of special interest. The supplement consists of

ammonium succinate, magnesium succinate, calcium succinate, zinc fumarate, glutamate, glycine, and vitamin E.

The main effect of the SBDSC that sets it apart from all other menopausal treatments is the regulation of the mitochondrial function (the key component of cellular metabolism) [25]. Mitochondrial dysfunction is the basis for a wide spectrum of clinical presentations [26, 27, 28, 29], many of which are similar to the symptoms of pathological climacteric.

Potential benefits of the SBDSC in menopausal symptoms therapy are achieved by re-establishing communications between central and peripheral components of the endocrine system, as the succinate anion acts both as a substrate and a regulator. Succinate provides energy supply and catecholamine-like influence on the hypothalamus, which increases the organ's sensitivity to input signals from peripheral endocrine glands [25].

Positive effects of the SBDSC have been demonstrated in a number of animal experiments, but only two clinical studies that showed positive effects of the SBDSC on menopausal symptoms had been conducted [30, 31]. The purpose of this randomized, placebo-controlled study was to evaluate the effectiveness and safety of the SBDSC in relieving vasomotor and psychosomatic symptoms in women during menopausal transition and postmenopausal period.

Methods

The study was conducted in accordance with the principles of the World Medical Association (Declaration of Helsinki) "Recommendations guiding physicians in biomedical research involving human subjects" and the National Standard of the Russian Federation "Proper Clinical practice".

The study enrolled women between 42 and 60 years of age, with vasomotor and psychosomatic complaints, in the late phase of menopausal transition (stage -1 according to the STRAW+10 classification) [32], i.e. those with delayed menstruation for at least 60 days and with FSH above 25 mIU/mL; and in postmenopause, i.e. those with amenorrhea for 12 months or more. All patients signed informed consent.

Patients were not included in the study if any of the following was present at the time of the screening: cancers of the reproductive system; extragenital cancers in remission for less than 5 years; conditions requiring hospitalization in the next 6 months; endocrine diseases (thyroid, adrenal, pituitary, hypothalamus) with abnormal hormonal secretion; surgeries of sex organs or breasts within one year or less of the screening; any surgeries done less than 3 months prior to

the screening; current HRT; psychiatric diseases; diabetes mellitus; taking other supplements that may affect climacteric syndrome within one month of enrolling in the study.

Based on the above criteria, 104 patients were enrolled in the study. Randomization with stratification to two equal groups was done in order to achieve balanced distribution at each of the study's centers. The randomization was done using an automated method of random number generation with values evenly distributed in the 0÷999 interval. The initial number for randomization was created by computer's pulse generator and is stored in case the results need to be recovered.

For all patients, the following data was collected: demographic, general health, general and gynecological medical history, menstrual function, and information on their climacteric period. Somatic diseases presence was determined based on the medical documentation presented by the patients Anthropometric measurements (height, body weight, waist circumference, BMI calculated using Ketle index – body weight, *kg*, divided by height, *m*, squared) and vital signs were taken; patients underwent general and gynecological exams. Standard laboratory evaluation included blood panels (general and biochemical), urinalysis. Mammogram and transvaginal sonography of lower pelvic organs were also used. For perimenopausal women, the sonography was done on day 5 after the onset of menstrual bleeding.

In order to evaluate patients' initial status and the effects of the SBDSC on the vasomotor and psychosomatic symptoms of the climacteric syndrome, the Greene Climacteric Scale [33, 34] and Spielberger-Hanin anxiety test were employed. The Spielberger-Hanin test is the only method that allows to differentially measure anxiety as a personal trait and as a state related to a certain situation. Reactive (situational) anxiety (SA) appears as a reaction to stress factors (stressors), often of socio-psychological character (expectation of an aggressive reaction, threat to self-esteem etc.). Personal anxiety (PA) describes the susceptibility of a person to some or other stressors based on the individual abilities. Actual anxiety (AA) is an integral indicator of anxiety levels and is the sum of SA and PA.

At the beginning and during the course of the study, blood levels of estradiol, follicle stimulating hormone (FSH), luteinizing hormone (LH), leptin, and apolipoproteins A_1 and B were measured. Hormone levels were determined by radio immune assays (RIA) using the following kits: estradiol, FSH, LH – RIO kit, IMMUNOTECH A.S., Czech Republic; leptin – RIA-1624, DRG instruments GmbH, Germany. Apolipoprotein levels were determined using kits manufactured by DiaSis Diagnostic Sistems GmbH, Germany.

After initial evaluation and randomization, patients in the treatment group received the following treatment: 2 capsules (one white, 200 mg, and one orange, 200 mg), once a day with a meal, for 3 months. Patients in the control group received placebo – capsules with high purity corn starch. Same capsules were used for placebo and SBDSC – odorless and flavorless, with the name of the SBDSC written on each capsule. Patients received a monthly supply of SBDSC /Placebo at each visit during the course of the study. The patients were asked to return any unused capsules to the study center at each visit (no returns were made).

Evaluation of changes of the above-mentioned parameters from the initial values was done monthly. For the entire duration of the study, the patients kept diaries, where they recorded their diets, any complaints, medications, and anything else they would consider important. Every two weeks a doctor-investigator contacted the patients by phone to inquire about their general wellbeing, complaints, and medications.

The data obtained during the study was analyzed using MSOffice 2010 and STATISTICA 12. Average (M), standard error (m) and deviation (σ), dispersion interval (minimum and maximum) were calculated for quantitative parameters. Frequency of qualitative parameters was expressed in percent (%). The data was initially processed using one-dimensional dispersion analysis. All measured parameters were evaluated for normal distribution using Shapiro-Wilk, Lilliefors, and Kolmogorov–Smirnov tests. In order to describe quantitative parameters with nonparametric characteristics the following were used: volume sample, median and average, maximal, minimal, and 95% confidence interval. Comparative analysis of effectiveness parameters (quantitative variables) was performed by comparing averages in the treatment and control groups. Comparison of averages for each parameter in the two groups was done using Mann-Whitney U-test (nonparametric statistics) or Student's t-test (for normal distribution). The Friedman test was used in dynamic analysis. For calculations of the Greene Climacteric Scale scores between groups, χ^2 test was used (recommended for evaluation of discrete samples). Differences were considered statistically significant when $p<0.05$ (95% confidence level).

Results

Assuming that some patients would dropout at the screening, 191 women were screened. 104 of them were randomized and 102 completed the study (52 in the treatment group and 50 in the control group). One patient in the control group withdrew due to a hypertensive emergency requiring re-evaluation of her blood pressure therapy, which could affect parameters under investigation; second patient withdrew due to a surgery.

The mean age of the study population was 51.54±4.21 years in the treatment group and 51.16±4.21 years in the control group, and did not differ significantly. Of the total 102 patients who completed the study, 44 (43.1%) were perimenopausal, and 58 (56.9%) were postmenopausal. At the enrollment, amenorrhea for perimenopausal women lasted from 3 to 11 months, and FSH varied from 37 to 72 mIU/ml

All patients included in the study complained about hot flashes and/or night sweats: only hot flashes – 23 (22.55%), only night sweats - 18 (17.65%), hot flushes and night sweats - 61 (59.8%). Besides vasomotor symptoms, the patients complained about physical and psychological symptoms that lowered their quality of life.

Medical documentation analysis and clinical evaluation showed various pathologies in 38 (73.1%) patients in the treatment group and in 40 (80%) patients in the control group. Among 35 (67.3%) patients in the treatment group 63 pathological conditions were registered: diseases of the cardiovascular system (30.2%), gynecological diseases (25.4%), disease of the gastrointestinal tract (19%) and skeletal system (6.4%). 53 pathological conditions were registered among 38 (76%) patients in the control group: diseases of cardiovascular system (37.7%), gynecological diseases (26.3%), disease of the gastrointestinal tract (5.7%) and skeletal apparatus (5.7%).

Breast tissues had fibroadipose involutions (17 and 11.5% in the treatment and control groups respectively) and benign breast changes (6.9 and 3.8% in the treatment and control groups respectively).

Main ECG pathologies were: CLC syndrome (5.8 and 2% in the treatment and control groups respectively), RSR pattern (5.8 and 2% in the treatment and control groups respectively); first degree AV block (1 patient in each group) and sinus bradycardia (3.9 and 12% in the treatment and control groups respectively).

Ultrasound evaluation showed that 11 (21.2%) patients in the treatment group and 11 (22%) in the control group had uterine fibroids of various sizes, and 5 (9.6%) patients in the treatment group and 6 (12%) patients in the control group had signs of adenomyosis.

Anthropometric parameters, results of the blood panels (general and biochemical) and urinalysis, levels of apolipoprotein A_1 and B, and leptin did not differ significantly between the two groups. Concentrations of estradiol, FSH, and LH also did not differ significantly between the two groups and corresponded to perimenopausal and postmenopausal statuses.

Results of the initial Greene test (Table 1) and Spielberger-Hanin test (Table 2) were similar between the SBDSC and placebo groups. Initial differences of the Greene Climacteric Scale scores were not statistically significant, despite fewer total complains in the control group.

The exception was «sadness and depression» symptom that occurred more frequently in the treatment group (p=0.0383).

The Greene Climacteric Scale was used to evaluate changes in 21 symptoms during the course of the study. The results of the test are presented in Table 1.

Table 1.

Greene climacteric scale results

	Group	N	Number of women with symptoms		
			Before treatment	After treatment	P
1. Heart palpitation	SBDSC	52	42 (80.8%)	30 (57.7%)	0.0265
	Placebo	50	31 (62.0%)	33 (66.0%)	> 0.05
	P		> 0.05	> 0.05	
2. Feeling tense or nervous	SBDSC	52	39 (75.0%)	25 (48.1%)	> 0.05
	Placebo	50	41 (82.0%)	42 (84.0%)	> 0.05
	P		> 0.05	0.0098	
3. Difficulty sleeping	SBDSC	52	46 (88.5%)	26 (50.0%)	< 0.0001
	Placebo	50	38 (76.0%)	44 (88.0%)	> 0.05
	P		> 0.05	< 0.0001	
4. Increased excitability	SBDSC	52	40 (76.9%)	29 (55.8%)	0.0086
	Placebo	50	38 (76.0%)	37 (74.0%)	> 0.05
	P		> 0.05	< 0.0001	
5. Panic attacks	SBDSC	52	21 (40.4%)	16 (30.8%)	> 0.05
	Placebo	50	21 (42.0%)	14 (28.0%)	> 0.05
	P		> 0.05	> 0.05	
6. Difficulty concentrating	SBDSC	52	32 (61.5%)	19 (35.5%)	0.0055
	Placebo	50	35 (70.0%)	32 (64.0%)	> 0.05
	P		> 0.05	0.0013	
7. Feeling tired or lacking energy	SBDSC	52	46 (88.5%)	24 (46.2%)	< 0.0001
	Placebo	50	42 (84.0%)	44 (88.0%)	> 0.05
	P		> 0.05	< 0.0001	
8. Loss of interest in most things	SBDSC	52	27 (51.9%)	19 (36.6%)	> 0.05
	Placebo	50	26 (52.0%)	24 (48.0%)	> 0.05
	P		> 0.05	> 0.05	
9. Sadness or depression	SBDSC	52	48 (92.3%)	23 (44.3%)	< 0.0001
	Placebo	50	35 (70.0%)	37 (72.0%)	> 0.05
	P		0.0383	0.0003	
10. Crying spells	SBDSC	52	30 (57.7%)	20 (38.5%)	> 0.05
	Placebo	50	32 (64.0%)	27 (48.0%)	> 0.05
	P		> 0.05	> 0.05	
11. Irritability	SBDSC	52	45 (86.5%)	25 (48.1%)	< 0.0001
	Placebo	50	36 (72.0%)	37 (74.0%)	> 0.05
	P		> 0.05	< 0.0001	
12. Dizziness or fainting	SBDSC	52	26 (50.0%)	19 (36.5%)	> 0.05
	Placebo	50	20 (40.0%)	13 (26.0%)	> 0.05

	P		> 0.05	> 0.05	
13. Feeling pressure or tightness in the head or other body parts	SBDSC	52	35 (67.3%)	21 (40.4%)	> 0.05
	Placebo	50	29 (58.0%)	28 (56.0%)	> 0.05
	P		> 0.05	> 0.05	
14. Numbness or tingling in some body parts	SBDSC	52	30 (57.7%)	26 (50.0%)	> 0.05
	Placebo	50	31 (62.0%)	28 (56.0%)	> 0.05
	P		> 0.05	> 0.05	
15. Headaches	SBDSC	52	47 (90.4%)	28 (53.9%)	< 0.0001
	Placebo	50	42 (84.0%)	40 (80.0%)	> 0.05
	P		> 0.05	< 0.0001	
16. Muscle and joint pain	SBDSC	52	44 (84.6%)	24 (46.2%)	< 0.0001
	Placebo	50	41 (82.0%)	32 (46.0%)	> 0.05
	P		> 0.05	0.029	
17. Numbness of hands and feet	SBDSC	52	19 (36.5%)	19 (36.5%)	> 0.05
	Placebo	50	15 (30.0%)	11 (22.0%)	> 0.05
	P		> 0.05	> 0.05	
18. Difficulty breathing	SBDSC	52	19 (36.5%)	14 (26.9%)	> 0.05
	Placebo	50	16 (32.0%)	14 (26.0%)	> 0.05
	P		> 0.05	> 0.05	
19. Hot flashes	SBDSC	52	48 (92.3%)	26 (50.0%)	< 0.0001
	Placebo	50	38 (76.0%)	39 (78.0%)	> 0.05
	P		> 0.05	0.0003	
20. Night sweats	SBDSC	52	43 (82.7%)	24 (46.2%)	< 0.0001
	Placebo	50	36 (72.0%)	37 (74.0%)	> 0.05
	P		> 0.05	0.024	
21. Lack of sex drive	SBDSC	52	41 (78.9%)	13 (25.0%)	< 0.0001
	Placebo	50	36 (72.0%)	38 (76.0%)	> 0.05
	P		> 0.05	< 0.0001	

Analysis of the results revealed that the 3-month course of SBDSC treatment significantly reduced the number and severity of complaints. There was a statistically significant decline in the frequency of heart palpitations complaints, with a complete disappearance of severe and moderate forms of the symptom; in the control group, no statistically significant improvement of this symptom was observed. Frequency of “difficulty sleeping” complaints decreased, with the severe forms of the symptom remaining in the single digits. Similar results were obtained for the «increased excitability» and «difficulty concentrating» symptoms. The treatment group showed significant improvement in frequency and severity of the psychological symptoms: “feeling tired or lacking energy”, “irritability” –with a complete disappearance of severe forms of these symptoms. The opposite was observed in the control group, where there was a tendency toward an increase in the frequency and severity of sleep and psychological symptoms’ complaints.

Among somatic symptoms, the biggest effect of SBDSC therapy was evident in headaches and muscle/joint pain, with no patients complaining about severe symptoms and a

significant decrease of moderate symptoms complaints by the end of the study. In the control group, the headaches increased in intensity overtime; there was an insignificant decrease in muscle and joint pain complaints. The frequency of vasomotor symptoms decreased significantly among women who received SBDSC by the end of the study, with no patients reporting severe forms of the symptoms; the frequency of moderate forms of the symptoms showed significant reduction. In the control group we observed negative dynamic of the vasomotor symptoms. Finally, there was an improvement in the sexual aspects of the climacteric syndrome, while no dynamic changes were observed in the control group.

Comparative analysis between treatment and control groups showed statistically significant differences in the following symptoms: «feeling tense or nervous», «difficulty sleeping», «increased excitability», «difficulty concentrating», «feeling tired or lacking energy», «irritability», «headaches», and «lack of sex drive». The depression symptom deserves a special note here: initially it was observed at a higher frequency in the SBDSC group, but after 2 months of therapy the situation changes dramatically – depression became more frequent in the control group. By the end of the study the differences between the two groups for depression symptom increased. Statistically significant differences in «muscle and joint pain» and «night sweats» between treatment and control groups were registered after 3 months of therapy. The fastest response to therapy was observed for hot flashes: after just one month of treatment the differences between the two groups reached statistically significant levels, and remained such until the end of the study.

The results of the Spielberger-Hanin test are shown in Table 2.

Table 2.

Spielberger-Hanin test results (averages*, median)

Anxiety	**Time**	**SBDSC n=52**	**Placebo n=50**	**p^1**
SA (situational anxiety)	Initial	-2.31±8.29*	-2.08±10.63*	> 0.05
	30 days	-4.96±7.22*	-2.04±8.27*	> 0.05
	60 days	-6.31±6.24*	-1.5±8.2	= 0.0010
	90 days	-8.25±5.84*	-4±8.16	= 0.0004
	p^2	< 0.0001	> 0.05	
PA (personal anxiety)	Initial	13.29±7.53*	13.16±9.34*	> 0.05
	30 days	10.87±6.91*	12.6±8.82*	> 0.05
	60 days	8.75±6.52*	11.5±12.7	= 0.0054
	90 days	6.31±5.65*	11±10.6	< 0.0001
	p^2	< 0.0001	> 0.05	
AA (actual anxiety)	Initial	45.98±14.27*	45.6±18.8*	> 0.05
	30 days	40.87±12.75*	45.66±15.95*	> 0.05
	60 days	37.44±11.34*	46.29±15.98*	= 0.017
	90 days	33.04±18.8*	45.5±8.17	< 0.0001
	p^2	< 0.0001	> 0.05	

* – average for normal distribution

p^1 – SBDSC vs Placebo

p^2 – Initial vs 90 days

The results showed that SA and PA in the treatment group began to decrease after just one month of therapy; this dynamic remained for the course of the study, with statistically significant differences compared to the initial evaluation. In the control group, SA and PA decreased only by the end of the study, but did not reach statistically significant differences compared to the initial evaluation. Statistically significant differences in the SA and PA between the two groups were seeing after 2 and 3 months of treatment. In the placebo group, AA remained unchanged during the entire course of the study. In the SBDSC group, anxiety decreased and was significantly different from the initial values starting at two month of treatment. Differences between the two groups became significant after two months and remained such until the end of the treatment.

A number of biochemical markers were evaluated during the course of the study, including concentrations of gonadotropins and estradiol in blood plasma (Table 3).

Table 3.

Hormone blood plasma levels (average for normal, median for non-normal distribution, ±SD)

Hormones	Time	SBDSC n=52	Placebo n=50	p^1
Estradiol, pg/ml	Initial	36.94±164.7	40.91±117.09	> 0.05
	30 days	45.57±404.8	39.74±149.4	> 0.05
	60 days	50.41±377.31	37.56±101.5	< 0.0001
	90 days	54.07±386.74	39.10±113.88	< 0.0001
	p^2	< 0.05	> 0.05	
FSH, mIU/ml	Initial	62.33±25.01*	62.86±35.87	> 0.05
	30 days	57.62±28.83*	59.92±34.84*	> 0.05
	60 days	53.49±25.35*	62.64±33.75*	> 0.05
	90 days	52.45±23.5*	62.47±33.36*	> 0.05
	p^2	< 0.05	> 0.05	
LH, mIU/ml	Initial	31.53±14.01*	28.98±17.19	> 0.05
	30 days	29.24±14.6*	28.31±13.12	> 0.05
	60 days	27.1±12.6*	27.6±13.7	> 0.05
	90 days	26.27±10.76*	26.9±19.37	> 0.05
	p^2	< 0.05	> 0.05	

* - median for non-normal distribution

p^1 – SBDSC vs Placebo

p^2 – Initial vs 90 days

In the treatment group estradiol blood concentrations increased to statistically significant levels by the 3rd month of therapy, while remaining unchanged in the control group. Differences between the two groups became statistically significant after 2 months of therapy. Levels of FSH and LH in the SBDSC group decreased to statistically significant levels by the end of the study ($p<0.05$), however, no significant differences were observed in gonadotropin concentrations between patients in the SBDSC and placebo groups.

Concentrations of leptin in the SBDSC group were characterized by a statistically significant reduction that approached normal levels (Table 4), while in the control group there was a trend toward increased concentrations of the hormone.

Table 4.

Blood leptin concentrations (Me±SD)

Hormone	Time	SBDSC n=52	Placebo n=50	p^1
Leptin, ng/mL (0.5-13.8)	Initial	15.25±8.99	14.20±7.23	> 0.05
	90 days	14.15±7.23	14.35±0.36	> 0.05
	p^2	0.0072	> 0.05	

p^1 – SBDSC vs Placebo

p^2 – Initial vs 90 days

Concentrations of apolipoproteins are reflective of atherogenic potential and the risk of developing cardiovascular complications. The levels of apolipoprotein A_1 (high density lipoprotein, HDL) and B (part of all lipoproteins, except HDL, and chylomicra) significantly decreased in the SBDSC group (Table 5), however, the B/ A_1 ratio, which is a marker for cardiovascular risk, did not increase in individual observations. In the placebo group, levels of apolipoproteins remained unchanged. No differences were observed in the dynamics of the apolipoproteins levels between groups.

Table 5.

Levels of apolipoprotein A_1 and apolipoprotein B in patients' blood (Me±SD)

Apolipoproteins	Time	SBDSC n=52	Placebo n=50	p^1
A_1, g/L (1.05-2.05)	Initial	1.66±0.6	1.65±0.4	> 0.05
	90 days	1.63±0.39	1.51±0.36	> 0.05
	p^2	0.0035	> 0.05	
B, g/L (0.5-1.3)	Initial	0.94±0.3	0.91±0.23	> 0.05
	90 days	0.89±0.23	0.91±0.2	> 0.05
	p^2	0.0375	> 0.05	

p^1 – SBDSC vs Placebo

p^2 – Initial vs 90 days

In the SBDSC group, there was a small but statistically significant ($p<0.05$) decrease in body weight, BMI, and waist circumference (Table 6). In the control group, there was a small but statistically significant dynamic increase of the above-mentioned parameters. There were no significant differences between absolute values between the two groups.

Table 6.

Weight, BMI, and waist circumference (mean*, median, ±SD) during the course of the study

Parameter	Time	SBDSC n=52	Placebo n=50	p^1
Weight, kg	Initial	78.02±12.12*	78±14.18	> 0.05
	30 days	77.42±11.91*	78±14.05	> 0.05
	60 days	75.81±11.02*	78±14.20	> 0.05
	90 days	74.92±10.97*	78.5±13.92	> 0.05
	p^2	< 0.05	< 0.05	
BMI, kg/m²	Initial	28.73±4.66*	28.3±5.91	> 0.05
	30 days	28.48±4.62*	28.15±5.84	> 0.05
	60 days	27.93±4.21*	28.3±5.88	> 0.05
	90 days	27.58±4.09*	28.55±5.78	> 0.05
	p^2	< 0.05	< 0.05	
Waist circumference, cm	Initial	90.81±11.98*	88.85±13.7*	> 0.05
	30 days	89.67±11.09*	88.86±13.4*	> 0.05
	60 days	88.42±10.45*	88.85±13.45*	> 0.05
	90 days	87.2±9.82*	89.73±13.57*	> 0.05
	p^2	< 0.05	< 0.05	

* - average for normal distribution.
p^1 – SBDSC vs Placebo
p^2 – Initial vs 90 days

When evaluating changes in weight, BMI, and waist circumference at 1-, 2-, and 3-month time points, the differences between the groups become clear (Table 7), and differences in the dynamic parameters reach statistically significant levels.

Table 7.

Changes in weight, BMI, and waist circumference 1, 2, and 3 month time points (Me±SD)

Parameter	Time	SBDSC n=52	Placebo n=50	p*
Weight, kg	0 — 30 days	- 0.56±1.97	0±0.91	0.0007
	0 — 60 days	- 2.21±2.3	0.51±1.21	< 0.0001
	0 — 90 days	- 3.1±3.27	0.7±1.6	< 0.0001
BMI, kg/m²	0 — 30 days	- 0.26±0.65	0.1±0.33	0.0003
	0 — 60 days	- 0.81±0.83	0.21±0.46	< 0.0001
	0 — 90 days	- 1.16±1.23	0.25±0.61	< 0.0001
Waist circumference, cm	0 — 30 days	- 1.13±4.17	0.02±1.87	0.0110
	0 — 60 days	- 2.38±4.77	0±1.96	< 0.0001
	0 — 90 days	- 3.61±4.99	0.88±3.87	< 0.0001

* p – SBDSC vs Placebo

The results of SBDSC's influence on anthropometric parameters and levels of apolipoproteins demonstrate not only the positive effects of the supplement, but also its potential safety.

Clinical and laboratory safety parameters had been evaluated during the course of the study. Blood pressure, heart rate, body temperature, and breathing rate of the patients remained in the normal ranges and did not differ significantly between the SBDSC and placebo groups. Blood panel results for the SBDSC group revealed a slight increase in the number of platelets, from $226.5x1^9$±57.55 to $229x10^9$±43.45/L (p=0.4995), which does not exceed the normal range; while in the control group there was an increase in the number of erythrocytes from $4.52x10^{12}$±0.34 to $4.62x10^{12}$±0.31/L (p=0.0117), which also remained within normal ranges and thus did not have any clinical significance. Urinalysis did not reveal substantial changes during the course of the study. Evaluation of the biochemical blood panel showed a slight decrease of the average levels of aspartate aminotransferase (AST) in the SBDSC group, from 38.36±14.7 to 35.7±11.14 unit/L (p=0.0011); no significant differences were observed between the two groups.

Ultrasound evaluation was conducted during the 3rd month of therapy. SBDSC had no effect on the endometrial thickness - by the end of the study this parameter was 4.79±2.65 mm in the SBDSC group and 4.27±2.81 mm in the placebo group. There were no differences in the endometrial thickness between groups and within each group compared to the initial evaluation.

No adverse reactions were observed in the SBDSC group during the course of the study. In the placebo, group two adverse reactions were registered (hypertensive crisis and ovarian cyst); both are unlikely to be related to the treatment.

Discussion

The climacteric symptoms considerably impair the quality of life and send approximately 80% of women in menopausal transition and postmenopausal period to seek medical care. The most pronounced and specific menopause symptoms are hot flashes [5, 7, 25] – sudden dilation of small blood vessels that result in feeling hot, accompanied by hyperemia, increased heart rate, and perspiration. Besides vasomotor symptoms (hot flashes and night sweats), women in menopause transition and postmenopausal period are often bothered by difficulty sleeping, depression, and various psychosomatic symptoms [4, 35].

The study's results showed that SBDSC supplementation (2 capsules, once a day) during the course of even the first month has positive effects on patients with menopausal symptoms. Based on the results of the Green Climacteric Scale analysis, patients' condition improved in the

following parameters: «difficulty sleeping», «difficulty concentrating», «feeling tired or lacking energy», «sadness or depression», «irritability», «headaches», «muscle and joint pain», «hot flashes», «night sweats», and «lack of sex drive».

SBDSC's mechanism of impact on climacteric syndrome is not entirely clear. The root of menopausal symptoms is the gradual decline of ovarian function that leads to hypoestrogenism; a decrease of estrogens in the blood is one of the main causes of the appearance of vasomotor complaints [36]. Estrogen deficiency likely leads to the dysfunction of the brain's neuronal networks that experience deprivation of sorts as the result of the lack of regular hormonal effects [37, 38].

SBDSC supplementation changes the hormonal profile of patients: after 2 months there was a significant increase in estradiol levels, which can partially explain positive effects of SBDSC on menopause symptoms.

Studies that modeled hypoestrogenic status have shown that estradiol effect on various tissues is directly related to its blood concentration. Higher concentrations of estradiol are needed in order to initiate endometrial growth, compared to the levels needed to maintain bone mineral density or neuroprojections or prevention of vasomotor symptoms [39, 40, 41]. Estradiol levels less than 80 pg/mL are likely to have therapeutic effects while remaining safe in terms of excessive proliferation. Therefore, the average estradiol levels of 54.07 pg/ml achieved in our study can be considered physiologically comfortable and safe.

Aging of the nervous system and decline in the hypothalamic sensitivity to peripheral hormone signals play a significant role in the formation of menopausal symptoms and explain the appearance of vasomotor symptoms in pre-menopausal women with normal estradiol levels [42]. However, other changes are taking place at the same time in other areas of the brain. Sex steroids affect psychological functions of the nervous system, shape the behavior, affect mood, learning, long and short term memory, and one's verbal abilities [43].

SBDSC treatment allowed for the stabilization of psychological status of the patients, as evidenced by the results of the Greene Climacteric Scale and Spielberger-Hanin test. The frequency of depression significantly deceased in women who took SBDSC; differences between the groups were also statistically significant. Analysis of the Spielberger-Hanin test data supported a statistically significant decrease in anxiety (SA and PA components), which speaks to an increase in patients' stress resistance and improvement in their adaptability. A statistically significant reduction in the integral indicator of anxiety (actual anxiety, AA) is evidence of the improved psychological status of the patients.

Increased levels of estradiol that resulted from SBDSC treatment can partially explain improvement in psychological status, but other mechanisms of the supplement actions on the moods likely exist as well.

A decline in the ability to adapt plays a major role in the development of dysfunctions of the CNS. Loss of adaptive abilities in any tissue is closely related to mitochondrial dysfunction, which results in lower ATP levels, increase in the ROS production, and activation of cell death mechanisms. Mitochondria are capable of launching different cell death programs: autophagy, apoptosis and necrosis. The program the choice depends on the number of open pores in the mitochondria and the release of cytochrome C, endonuclease G and apoptosis-inducing factor, inhibitors of anti-apoptotic proteins, and caspase precursors. Autophagy is activated when a minimal number of pores are open, apoptosis – with half number of open pores, necrosis – with maximum number of open pores [44, 45]. Different programmed cells deaths cause differences in the tissues' functions, and deviations during these processes can lead to activation of inflammation, hypertrophic, and fibrous changes, which in turn will cause secondary dysfunctions of organs. Thus, mitochondrial dysfunction, which results from external factors, realizes its pathophysiological potential and disrupts functions and structures of tissues.

Mitochondrial dysfunction is closely associated with brain dysfunctions and is the foundation of neurodegenerative diseases, frequency of which increases in postmenopausal women [46, 47]. Moreover, cells with damaged mitochondria have a different response to estrogen signaling [48], forming negative clinical effects instead of expected positive ones, which is described in trials on MHT. Increased estradiol levels and improved mitochondrial function that occur during SBDSC therapy, allow speculating about the high degree of safety and expected positive outcomes. We can also speculate that treatments targeting mitochondrial dysfunctions will have positive effects on both psychological health and Alzheimer's risk.

Stabilization of body weight and prophylactics against obesity take a special place in management of menopausal women. The problem of weight gain in women during peri- and postmenopausal periods is well known [49]. It is related to the age-related increase in insulin resistance and general changes in the hormonal system of the body [50]. Increase in the fat tissue and associated with it disruptions in fat and carbohydrate metabolism are serious risk factors of cardiovascular diseases and cancer [51, 52, 53]. The cellular component of pathophysiological relationship between metabolic disorders and socially significant diseases, however, is often underestimated. Meanwhile, developing dyslipidemia, hyperglycemia, inflammation, ischemia, and tissue damage initiate secondary mitochondrial dysfunction [44, 54], which has an immediate and detrimental effect on tissues' functions.

In our study, SBDSC supplementation led to statistically significant weight loss and decrease in BMI and waist circumference. Dynamic analysis of these parameters in the two groups revealed the decrease starts at 2 months of SBDSC treatment; in the placebo group, the weight remained stable. Weight loss in SBDSC group occurs due to fat tissue reduction, which is indirectly shown by a statistically significant, albeit a small, decrease in leptin levels – a hormone produced by adipocytes [55]. This decrease can be explained by other mechanisms as well, which have potentially positive effects. Increase in leptin levels beyond normal is known to raise tissues' resistance to the hormone, resulting in its functional deficit. Leptin has many metabolic effects. It directly acts on tissues, improving their insulin sensitivity [56, 57], and also influences orexigenic (neuropeptide Y, Agouti-related peptide) and anorexigenic (α-melanocyte-stimulating hormone) neuropeptides of the brain, which modifies eating behavior, suppressing appetite and lowering food intake [58, 59]. Regardless of the mechanism of action, weight loss that is connected to the reduction of fat tissue has health benefits in terms of cardiometabolic prevention. Therefore, our data suggests additional positive effects of SBDSC – a decrease in cardiometabolic risk factors.

Conclusions

1. Based on the results of the study, we can state that SBDSC treatment significantly decreases frequency and intensity of menopausal symptoms, such as difficulty sleeping, increased excitability, difficulty concentrating, feeling tired or lacking energy, sadness or depression, irritability, headaches, muscle and joint pain, hot flashes, night sweats, and lack of sex drive.

2. SBDSC supplementation increases patients' resistance to stress and adaptive abilities of the nervous system, which is evidenced by the decreases in situational, personal, and actual anxieties.

3. SBDSC treatment results in a statistically significant increase in estradiol blood levels to the physiologically comfortable levels, which do not cause proliferation of the endometrium.

4. Women who took SBDSC showed a statistically significant decrease in body weight, BMI, and waist circumference. This likely occurred via reduction in visceral fat, which is indirectly supported by a significant decrease in leptin levels.

5. Comparative analysis of vital signs, blood and urinalysis did not reveal significant differences after SBDSC treatment compared to placebo, which leads us to conclude that SBDSC is a safe dietary supplement.

6. Based on the results of the study we conclude that women with climacteric syndrome can take SBDSC supplement as follows: 2 capsules, once a day with a meal, preferably in the morning, for 3 months.

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