Title: ”The Effect of Standardized Withania Somnifera Extract on Endocrine, Metabolic, and Psychometric Parameters, in Women With Polyendocrine Metabolic Ovarian Syndrome: a Randomized Active-controlled Trial”.

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Type of research study: a randomised, active-controlled intervention trial.

Aim and rationale of the study: to evaluate the impact of supplementation with a standardised extract of *Withania somnifera* on endocrine, metabolic and psychometric parameters in women diagnosed with polyendocrine metabolic ovarian syndrome (PMOS). *Withania somnifera*, commonly known as Ashwagandha, is a medicinal plant that has been shown to potentially contribute to the regulation of hormones, specifically by modulating the biosynthesis of luteinising hormone (LH) and follicle-stimulating hormone (FSH). As demonstrated in studies, it may also reduce insulin resistance and alleviate neuropsychiatric symptoms such as anxiety disorders, depressive disorders and insomnia. The aforementioned effects have the potential to be advantageous for women diagnosed with PMOS. The study will be conducted in a group of female patients aged between 18 and 45 years who have been diagnosed with polyendocrine metabolic ovarian syndrome.

Total number of participants: 28 women.

Study group: patients attending the Endocrinology Outpatient Clinic of the Central Clinical Hospital of the Medical University of Lodz who had been diagnosed with PMOS according to the Rotterdam criteria and who were not taking any medication with a documented effect on mood or concentration, nor any dietary supplements.

Study group size: 14 women.

Control group: patients from the Endocrinology Outpatient Clinic of the Central Clinical Hospital of the Medical University of Lodz, diagnosed with PMOS according to the Rotterdam criteria, not taking medication with a documented effect on mood and concentration or dietary supplements, and not receiving Withania somnifera supplementation.

Control group size: 14 women.

Timeframe of the intervention: 3 months.

The duration of the follow-up: 6 months commencing from the enrolment of the final patient in the study.

The number and timing of measurement points: 3 assessment points were to be conducted before the commencement of the intervention, 3 months after the commencement of the first set of supplement intake, and 3 months after the commencement of the second set of supplement intake. These were to be assessed individually for each study participant.

Intervention:

a) study group:
   • *Withania somnifera* at a dose not exceeding 10 mg of withanolides per day,
   • cholecalciferol at a dose not exceeding 2.000 IU per day, except for patients in whom a deficiency has been diagnosed requiring a higher dose,
   • folic acid at a dose of 0.4 mg per day,
   • 5-methyltetrahydrofolate (5-MTHF) at a dose of 0.4 mg per day,
   • nutritional education in the form of online lectures.

a) control group:
   • cholecalciferol at a dose not exceeding 2.000 IU per day, except for patients in whom a deficiency has been diagnosed requiring a higher dose,
   • folic acid at a dose of 0.4 mg per day,
   • 5-methyltetrahydrofolate (5-MTHF) at a dose of 0.4 mg per day,

• nutritional education in the form of online lectures.

Investigative tools:

a) blood biochemistry tests

- complete blood count,
- lipid profile (total cholesterol, TG, HDL-C, LDL-C),
- fasting blood glucose and insulin levels; these will be used to calculate the HOMA-IR and QUICKY indices, which are used to diagnose insulin resistance,
- C-reactive protein (CRP) levels in the blood.
- total testosterone levels in the blood,
- luteinising hormone (LH) levels in the blood,
- follicle-stimulating hormone (FSH) levels in the blood,
- prolactin (PRL) levels in the blood,
- sex hormone-binding globulin (SHBG) levels in the blood,
- dehydroepiandrosterone sulphate (DHEA-S) levels in the blood,
- alanine aminotransferase (ALT) levels in the blood,
- cortisol levels in the blood,

b) measurement of blood pressure using an upper-arm sphygmomanometer,

c) anthropometric measurements – measurement of waist circumference, hip circumference, body weight and height, used to calculate the WHR and BMI,

d) body composition analysis using the bioelectrical impedance analysis (BIA) method,

e) questionnaires administered via an online survey (Computer-Assisted Web Interview, CAWI) by sending a personalised invitation by email, together with a link to the questionnaire

- Beck Depression Inventory (BDI-II),
- Perceived Stress Scale (PSS-10),
- Pittsburgh Sleep Quality Index (PSQI),
- International Physical Activity Questionnaire-Short Form (IPAQ-SH),
- Food Frequency Questionnaire (FFQ-6),
- a proprietary questionnaire concerning socio-demographic characteristics and containing specific questions (comorbidities, medications taken).

Venue and method of participant recruitment: the Endocrinology Outpatient Clinic at the Central Clinical Hospital of the Medical University of Lodz. The recruitment process will be overseen by the Head of the clinical arm of the study, in collaboration with the Contractor. All patients undergoing examination at the aforementioned Centre will be referred to the Endocrinology Outpatient Clinic of the Central Clinical Hospital of the Medical University of Lodz. The documentation obtained will be anonymised.

Criteria for allocation to the groups: random allocation ensured through the use of a randomisation process, independent of the preferences of the study participants and the Head of the clinical part of the study.

Inclusion Criteria:

- a diagnosis confirming the presence of polycystic ovary syndrome,
- age falling within the closed range of 18–45 years,
- absence of pregnancy confirmed by measurement of human chorionic gonadotrophin (β-hCG) levels in serum or urine, or by a gynaecological examination,
- not currently breastfeeding,
- no implanted metallic devices, such as: prostheses, pacemakers, defibrillators, stents, or metallic sutures in the heart or blood vessels,
- no diagnosed epilepsy and no history of epileptic seizures,
- not taking anti-epileptic medication,
- not taking benzodiazepine-based sedatives,

- not taking medicines containing metformin (e.g. Avamina, Formetic, Glucophage, Metformax, Siofor),
- not taking preparations containing oestrogens and/or progestogens,
- not taking dietary supplements.

Exclusion Criteria:

- no diagnosis confirming the presence of polycystic ovary syndrome,
- age under 18 or over 45,
- pregnancy confirmed by measurement of human chorionic gonadotrophin (β-hCG) levels in serum or urine, or by a gynaecological examination,
- currently breastfeeding,
- having implanted metallic devices, such as: a prosthesis, a pacemaker, a defibrillator, a stent, or a metallic suture in the heart or a blood vessel,
- diagnosed epilepsy or a history of epileptic seizures,
- taking anti-epileptic medication,
- taking benzodiazepine-based sedatives,
- taking medicines containing metformin (e.g. Avamina, Formetic, Glucophage, Metformax, Siofor),
- taking preparations containing oestrogens and/or progestogens,
- poor general health, severe generalised and systemic diseases, including liver and kidney failure, malabsorption syndromes,
- allergy to plants of the nightshade family,
- taking dietary supplements.

Probability of subjects gleaning personal advantages from involvement in the initiative: participants in both the study and control groups will acquire knowledge regarding their health status. It is imperative to emphasise that the results of the 3 rounds of blood tests, in conjunction with the findings derived from the methods employed to assess nutritional status, including anthropometric measurements and body composition analysis utilising the BIA method, are pivotal in comprehending the subject's health status. Each participant in the study will receive a printout from the body composition analyser, and all test results will be discussed with the patients using language tailored to their individual cognitive abilities, and therefore in a way that is understandable and leaves no room for doubt. Furthermore, all study participants will be required to attend lectures on the topic of proper nutrition in the context of polyendocrine metabolic ovarian syndrome. During these sessions, time will be allocated for discussion, during which patients will have the opportunity to pose individual questions to the clinical dietitian. All women participating in the study will also benefit from receiving dietary supplements containing cholecalciferol and folic acid in appropriate doses, the use of which is in line with the recommendations for the Polish population and the guidelines for women during the preconception period.

Statistical analysis plan: the evaluation of the study results will be conducted using STATISTICA version 12.0 or 13.1 (Tibco Software Inc., Palo Alto, CA, United States). The analysis of qualitative and quantitative data will be conducted through the utilisation of appropriate statistical tests, contingent upon the distribution of the variables under investigation.