herbmed.skums.ac.ir

Promising Effects of Vitex Agnus-Castus on Quality of Life and Sexual Function in Premenopausal Relapsing Remitting Multiple Sclerosis Patients

Sara Rahgooy[1], ,Sepideh Arbabi Bidgoli[2]*, Saeed Shahbeigi[3], Shima Tavakol[4], Mohammad Hossein Salehi Surmaghi[5]

[1] Faculty of Pharmacy and Pharmaceutical Sciences, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran

[2] Department of Toxicology and Pharmacology ,Faculty of Pharmacy and Pharmaceutical Sciences ,Islamic Azad University, Tehran Medical Sciences (IAUTMU),Tehran-Iran.

[3] Loghman Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[4] Cellular and Molecular Research Center, Iran University of Medical Sciences, Tehran, Iran

[5] Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

Received: 15 November 2020 Accepted: 6 July 2021

Original Article

Abstract:

Background and aims: Current therapeutic regimens are not able to solve hormonal imbalance in young multiple sclerosis (MS) women and the clinical benefits of hormone therapy in MS patients remain controversial. This open labeled pilot study was aimed to find the role of *Vitex agnus-castus* plant supplement daily regimen in serum testosterone level , Expanded Disability Status Scale (EDSS), sexual function and overall quality of life in premenopausal MS patients.

Methods: To achieve these goals, Molecular Docking on *Vitex agnus-castus* standard fruit extract constitutes performed and 30 eligible patients were selected for the clinical

*Corresponding author: Department of Toxicology and Pharmacology ,Faculty of Pharmacy and Pharmaceutical Sciences ,Islamic Azad University, Tehran Medical Sciences (IAUTMU),Tehran-Iran. Tel: +21-88003470. E-mail address: sepideharbabi@gmail.com

part of this study. Patients received two Vitagnus® film tablets in addition to their drugs for 90 days.

Results: Some plant extract components showed strong affinity to Androgen Receptor Ligand binding domain. Significant reduction in EDSS (1.839 vs.1.643, p=0.003) and significant elevation in serum testosterone levels [388.6(±0.16900) vs. 468. (±0.18144) 2ng/dL (p=0.026)] were also observed at the completion of the intervention. Menstrual irregularities disappeared in all 28 patients who completed this study. MSQL-54 criteria at the completion of study (day 91) showed lower stress levels, improved vitality/emotional health and normalized sexual activities without significant effect on sleep disorders and cognitive functions of patients. Vitex agnus-castus treatment by predictable AR LBD affinity improved the emotional state, sexual life, EDSS score and overall quality of life in premenopausal RRMS patients.

Conclusion: Our results suggest Vitagnus® film tablet as safe and promising supplement with some benefits in MS women who are suffering from disease induced emotional and sexual impairments.

Keywords: Multiple sclerosis, Vitex agnus-castus, Quality of life, Testosterone, Molecular docking

INTRODUCTION

Multiple sclerosis (MS) is an immune mediated neurodegerative disease of the central nervous system (CNS) with unknown etiology, increased risk of morbidity and comorbidity and sexual dimorphism (1). Different substantial gaps in MS related epidemiological and therapeutically knowledge requires more studies to investigate the contribution of gender, age, ethnicity and other underlying factors on MS incidence, on MS prevalence and to achieve more individual therapeutic and rehabilitation interventions (2). This hard-to-treat demyelinating disease is often diagnosed in the second or third decade of life and decreases the quality of life by inducing progressive disability. Diagnosis of MS is associated with lifetime substantial costs to the patients and healthcare systems as well as a wide range of psychological, social and cultural difficulties (3).

Lifestyle and environmental factors are key predisposing factors for the risk of MS in people with genetic predisposition to MS (4) but gender, age at diagnosis and other demographic specification act as key factors in disease phenotype, lifestyle changes and quality of life of patients (5). Epidemiological studies have also indicated higher suicide attempts (approximately twice) as a self-destructive behavior in recently diagnosed MS patients compared with the general population with higher incidence in patients with psychological disorders, impaired sexual life,

depression, social isolation and tendency for substance abuse (6); therefore, more attention should be paid to supplementation and integrative health for this large group of patients worldwide. Iranian MS women and other women in neighboring countries with similar social and cultural characteristics may suffer from destroyed sex life under masks without necessary sexual support from their partners or during rehabilitation process (7).

This neurodegenerative disease precipitates in patterns of relapsing-remitting (RRMS) or progressive-onset but RRMS shows a sex-bias and develops 3–4 times more frequently in premenopausal women with peak onset after puberty (8) which suggests the extensive involvement of sex hormones in RRMS progression (9). Basic knowledge on the contribution of sex hormones in disease susceptibility and management has been applied as "bedside to bench to bedside" approach and lead the health practitioners to newer gender specific treatment insights. Testosterone and estrogens demonstrate anti-inflammatory effect but testosterone has more neuroprotective effects by stimulating myelin formation in glutamate-induced neurotoxicity and oxidative stress and makes regeneration of nerve cells through binding to neural androgen receptor (AR) (Collongues N, 2018). Among sex hormones and gender specific mediators, downregulating role of testosterone in the systemic immune response by cell type-specific effects in the context of immunological disorders has also been considered before this new approach to testosterone hormone (10). We showed decreased serum testosterone levels in Iranian premenopausal MS patients compared to their age-matched healthy counterparts and the contribution of serum testosterone levels to underlying factors and disease progression. It seems that study on preventive role of sex hormones balance in disease progression and sexual life as key factors in quality of life and social/familial health of patients (12) can assist medical and rehabilitation teams in controlling diseases and improving the life satisfaction of MS patients.

Vitex agnus-castus (VAC) known as Chaste tree is a historical medicinal herb from the Verbenaceae family which grows in Eastern Mediterranean region and Central Asia and has been used for centuries to treat premenstrual syndrome and premenstrual dysphoric disorder. Many compounds including flavonoids,iridoid glycoside , p-hydroxybenzoic acid, alkaloids, essential oils, fatty oils, diterpenoids and steroids have been identified in the chemical analysis of Vitex agnus-castus(13). Animal studies on hydroalcoholic VAC extract showed the androgenic (14) and osteoprotective effects in male mice by preserving the cortical and trabecular bone as a safe alternative to Hormone

Replacement Therapy (HRT) (15). Dopaminergic effects of VAC extract (16)and its additional pharmacological actions via mu-opiate receptor have been already described (17) but pharmacological studies on its binding capacities to Androgen Receptor (AR) are still missing. Therefore, we investigated the binding capacity of standard VAC to AR using an in silico model and the possible effects of authorized VAC tablets (Vitagnus®) on serum testosterone levels, EDSS, sexual function and overall quality of life in a pilot open labeled study in premenopausal MS patients.

Methods

Structure-based virtual screening of VAC by Molecular Docking:

Before starting clinical interventions on Vitagnus® tablets, molecular docking was done to perform the structure-based virtual screening of VAC to Androgen Receptor (AR). For this aim, the main VAC components in standardized fruit extract were collected from two Database (RCSB-PDB and PubChem) and described by ID codes (Table 1). To detect the total affinity of VAC main constitutes to human AR as possible novel androgen receptor ligands. AR LBD was performed and compared with natural human main androgen (testosterone) . All necessary experiments performed by Molegro virtual docker v.6 (CLC Bio company, Aarhus, Denmark) software following protein and molecule preparation by 100 runs (18). Two different files (PDB and SDF) were provided by AR LBD (2PNU) analyzer (19) and investigated by Molegro molecular viewer V2.5 (Fig 1, A, B, C).

***Vitex agnus-castus* (VAC):**

Vitagnus® film tablet with Iran Registration Code (IRC) of 1228091827 were obtained from Poorsina Pharmaceutical Company in Tehran as gift. Each 320 mg tablet contained 200 mg of fruit extract of Vitex agnus-castus. Total fruit extract contains essential oil compounds including beta-cineole, α-pinene, sabinene, limonene and pinend, flavonoids including casticin, isovitexin and orientin, iridoids and glycosides but aucubin is the main component which should be standardized in VAC pharmaceutical preparations worldwide. Each Vitagnus® tablet contained 2.1 to 3.3 mg aqupin according to validated HPLC method of analysis and global standards of VAC derived pharmaceutical dosage forms (20) .

Clinical Studies

Study population and design:

To avoid any misdiagnosis, to achieve maximum homogeneity in the study population, and to provide the necessary groundwork for successful outcomes, we decided to select relapsing remitting MS patients (RRMS) from other subgroups of MS patients. To this end, we conducted an open labeled study on accessible RRMS candidates based on ethical issues. This

study initiated by women who referred to Dr. Shah Beygi's different Neurology Centers (Aghdasiye /Tehran North and Narmak/Tehran East). These patients referred to these clinics for diagnosis and treatment of neurological and movement disorders from March 2016 to March 2018 and diagnosed with RRMS and we enrolled accessible patients and follow up facilities in these clinics. After careful analysis of medical history, diet, smoking habits, therapeutic regimens and lifestyle factors in RRMS premenopausal women, a fraction of volunteers were enrolled in the study according to inclusion and exclusion criteria and final agreement of collaborating Neurologists according to the McDonald protocol.

Diagnosis of MS and ethical considerations:

Final diagnosis was confirmed based on available evidence (21) after at least one year from RRMS diagnosis and receiving their standard prescriptions. Participants followed their routine treatment during this additional intervention but they were finally selected based on their recorded diagnostic criteria, approval of collaborating gynecologist and endocrinologist and lack of hepatotoxicity, nephrotoxicity or other underlying diseases. All participants in this study provided written informed consent to participate in the study. The study protocol was approved by the ethics committee of Islamic Azad University, Tehran Medical Sciences University (code: IR.IAU.PS.REC.1396.169) on December 20, 2017.

Expanded Disability Status Scale (EDSS)

EDSS measures the physical disability of MS patients and was introduced by John F. Kurtzke (22). In the present study, the EDSS score, as a primary endpoint revealing disease progression and neurological impairment, was considered as one of the inclusion criteria. EDSS scores were compared before and after three months of treatment with Vitagnus® tablets in MS patients by appropriate statistical tests.

Data collection

Face-to-face interviews based on combined structured questionnaires of life style and Multiple Sclerosis Quality of Life questionnaire [(MSQOL)-54] were used to obtain the necessary data from participants. The structured lifestyle questionnaire contained sociodemographic, lifestyle and dietary information and filled out with the help of trained blinded staff at enrollment. MSQOL-54 included age, weight, height, BMI, education levels, family history of MS, history of gestational disorders , history of liver and kidney diseases, occupational status, second

hand or active smoking (>30 min /day), physical activity, living and working address (urban or rural areas), dietary habits, occupational exposure to heavy metals, and exposure to household chemicals. The heights and weights of volunteers were measured at enrollment and patients with BMI>28 were excluded. To complete MSQOL-54, standard questions regarding the general health, disease improvement, pattern of physical activity, physical health, movement disorders, emotional problems, social activity, body pain, feeling, health in general, cognitive function, sexual function, sexual interest, sexual satisfaction ,bowel and bladder function and "Best Possible Quality of Life" were asked and described by "Terribly Unhappy, Mostly dissatisfied, Mixed about Equally Satisfied and Dissatisfied, Mostly Satisfied, Pleased and Delighted" .

Inclusion Criteria

Volunteering RRMS patients who signed the written informed consent were included in the study after completing the necessary face-to-face interview. They were 18- to 45-year-old women born and living in Tehran with expanded disability status scale (EDSS) score of 0- 6.0 with at least one year passing from their first diagnosis and treatment.

Exclusion Criteria

RRMS patients with EDSS>6 at baseline, patients with early menopause (<45), history of other underlying diseases (e.g., different types of cancer or certain underlying or limiting diseases), lactation, MI>28, exacerbation of the disease within the last three months, corticosteroid use in the previous three months, change of immunomodulatory drugs during the last three months, simultaneous use of sex hormones and oral contraceptives (OCPs), cabergoline, bromocriptine, and spironolactone were excluded from the study. Accidental pregnancy, pruritis and poor adherence were the main reasons for excluding two patients on days 21 and 56 and the remaining 28 patients completed this 90-day study.

Study Design

The protocol of this open-label randomized clinical trial was designed by the investigators in 2017 and patients were enrolled from January 2018 to March 2019 after ethics committee approved the study protocol. Study size was determined by accessible patients in two clinics who met our inclusion criteria and completed the 3- month intervention. All study enrolled patients completed this study through following procedure:

1- Blood samples were collected from all participants and serum testosterone levels were determined before the main experiment;

2- Regardless the baseline serum Testosterone levels, Vitagnus® oral tablets were prescribed after careful physical examinations by neurologist and face to face interview by expert blinded staff. Patients filled out MSQOL-54 during monthly visits;
3- Each patient administered Vitagnus® pills two times daily for 90 days with online access to trained staff and service for reporting adverse reactions;
4- At the completion of the intervention, blood samples were undertaken again from 28 patients who completed the study; and
5- Vitagnus® adverse effects were followed up by daily online service and facilities. All adverse effects in the first week of study including gastrointestinal symptoms, menstrual irregularities, and erythematous rash were improved through patient adherence in the second week of study.

Serum testosterone levels

To determine the serum concentrations of Testosterone, blood samples were collected according to standard protocol and the serum was separated by cold centrifuge and collected in microtubes. Testosterone levels were compared with control by Chemiluminescence Immunoassays (CLIA) using cobas E411 (Roche Company) with serial number of 15D3-16 (22, 23).2.5. A commercial rat serum pool (catalog no. M5905; Sigma Chemical Co., St. Louis, MO) was spiked with various hormone concentrations, and recovery percentage and parallelism to the assay standard curve were determined. For each assay, the serum pool was spiked with testosterone across the assay range or vehicle to determine endogenous hormonal levels in the pool. Samples were run in duplicate, and each assay was repeated to confirm the results. Hormonal recovery from each spiked sample was determined by subtracting hormone values in vehicle-spiked controls from hormone spiked samples. The same procedure was repeated on the serums of age-matched healthy premenopausal women who referred to the same laboratory for annual physical exam (n=30). The control group showed normal Testosterone levels in their check -up visits history without any classic symptom of hyperandrogenism or other hormonal abnormalities.

Statistical analysis

The baseline characteristics between cases and control groups were compared using student t-test or Wilcoxon signed-rank test for parametric and non-parametric variables respectively, which were expressed as mean (±SD) for parametrical distribution or median (interquartile range, IQR) for non-parametrical distribution, and chi-square test for categorical variables, which were expressed as number (percentage) unless otherwise indicated. All data were analyzed using SPSS for Windows (version 23; IBM® SPSS® Statistics, Armonk, NY, USA) and a two-sided $p<0.05$ was considered significance level.

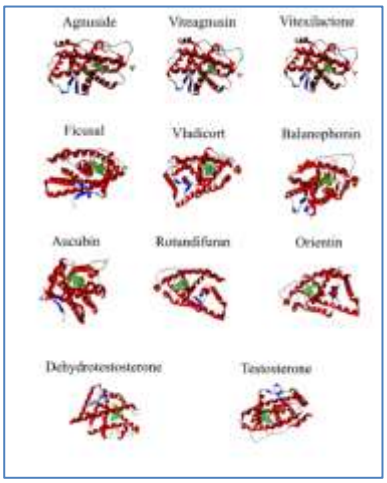
Table 1: MolDock scores of VAC constituents compared with standard androgen receptor ligands (Testosterone and dihydrotestosterone) described by Androgen Receptor Ligand Binding Domain(AR LBD).Standard VAC constituents of number 1 to 9 including agnuside, viteagnusin, vitexilactone, fcusal, vladicort, balanophonin, aucubin, rotundifuran and orientin were considered more potent that standard AR Ligands (highlighted by black) and aucubin as the main constitute of our pills (highlighted by red) was considered more potent than 2 main standards (-119.826 vs. .214 and -109.736 MolDock Scores) .

No.	Constitute	PubChem (CID)	MolDock Score
1	Agnuside	442416	-162.006
2	Viteagnusin	73349491	-132.96
3	Vitexilactone	21636178	-129.718
4	Ficusal	10496641	-128.181
5	Vladicort	5876	-125.007
6	Balanophonin	23252258	-121.841
8	Rotundifuran	9841926	-118.453
9	Orientin	5281675	-113.905
10	Dihydrotestosterone	13308	-111.214
11	Testosterone	6013	-109.736
12	Vitexin	5280441	-108.876
13	Casticin OR Vitexicarpin	5315263	-105.844
14	3-Epi-maslinic acid	25564831	-104.304
15	4,10-Aromadendranediol	14312736	-103.445
16	Luteolin	5280445	-101.593
17	3,7'-Dimethylquercetin	5280417	-101.313
18	Eupatorin	97214	-100.367
19	Penduletin	5320462	-98.146
20	3-O-Methylkaempferol	5280862	-97.93
21	Spathulenol	92231	-97.3991
22	Apigenin	5280443	-96.8511
23	Kaempferol	5280863	-94.3094
24	Epimanoyl Oxide	6432025	-94.2075
25	Ferulic acid	445858	-90.7666
26	p-Coumaric acid	637542	-78.5639
27	4-Hydroxybenzoic acid	135	-67.685
28	α-terpineol	17100	-64.393
29	Glyceryl linoleate (1-Monolinolein)	5283469	943.816

Fig. 1. Virtual binding capacity of VAC constituents *to ARLBD* by Molegro virtual docker v.6 (CLC Bio Company, Aarhus, Denmark) software following protein and molecule preparation by 100 runs. PDB and SDF files were provided by AR LBD (2PNU) analyzer . Figure *1A shows the location of the highest MolDock score VAC constituents in the cavity and indicated all components are locaed in the active site of LBD. Fig 1B shows the interaction of VAC constituents with AC LBD; and Fig 1C shows that amino acid residues of aucubin and anguside involved in the interaction with AR LBD. Blue bonds represent hydrogen interaction, while red color bonds represent steric interactions*

Fig1A ***Fig 1 B***

.



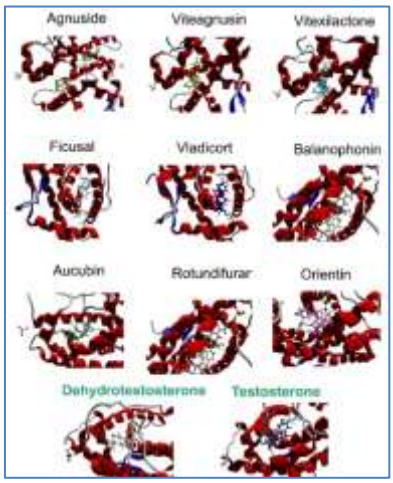
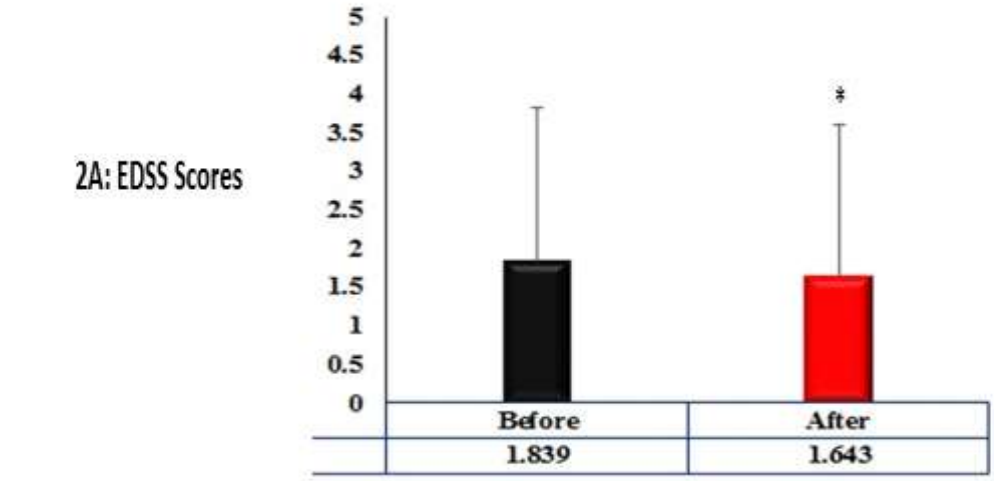


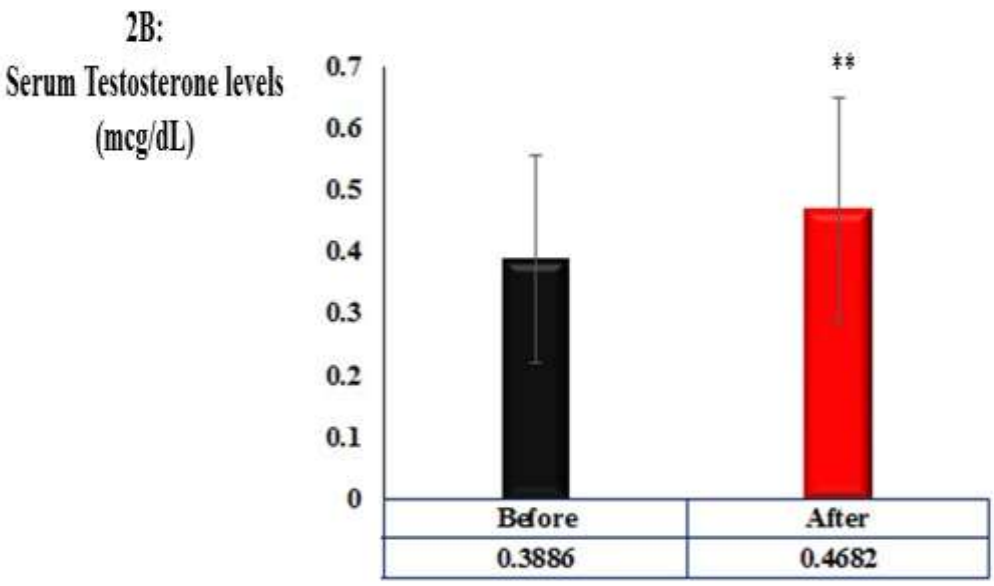
Fig1C

Table 2. Demographic information of patients

Underlying factors	**Mean (±SD)**	
Age (yr)	**32.36±6.64 (20-45)**	
Body Mass Index	**24.23±2.74 (23.2-27.8)**	
EDSS	**1.839±1.09(0.1-6)**	
Socioeconomic and underlying factors		
	Categories & Frequency (Number)	**Percentage (%)**
Academic Education (yes)	**23**	**82.14 %**
Home/occupational Exposure to Chemicals (yes)	**4**	**14.2%**
Employment (yes)	**11**	**39.3%**
Active/Second hand Smoking (yes)	**11**	**46.4%**
Reproductive and Sexual History		
Marriage (yes)	**19**	**67.8%**
History of Pregnancy (yes)	**12**	**42.9%**
History of Abortion (yes)	**3**	**10.7%**
History of Polycystic Ovary Syndrome (yes)	**6**	**21.4%**
History of Oral Contraceptive intake (yes)	**12**	**42.9%**
Menstrual Irregularity (yes)	**16**	**57.1%**

Fig. 2. Evaluation of MS Clinical (EDSS) and hormonal (Testosterone) following three months oral regimen of Vitagnus® tablets (N = 28). Fig 2A shows significant decrease in EDSS (1.839 vs. 1.643, p=0.003) and Fig 2B indicates changes in serum testosterone levels (p=0.026).





Results

VAC standard constituents and Aucubin molecular docking

Molecular docking was performed to investigate the interaction of VAC standard constituents with AR LBD (Table 1). Our results showed that the MolDock score of aucubin (the standardized component of Vitagnus® tablet) and the other constituents including agnuside, viteagnusin, vitexilactone, ficusal, vladicort, balanophonin, rotundifuran, orientin, and dihydrotestosterone were more potent than testosterone and dihydrotestosterone to AR LBD, respectively. Notably, the MolDock score of the following constituents gradually decreased compared to testosterone and dihydrotestosterone; vitexin, casticin (vitexicarpin), 3-epi-maslinic acid, 4,10-aromadendranediol, luteolin, 3, 7-dimethylquercetin, eupatorin, penduletin, 3-O-methylkaempferol, spathulenol, apigenin, kaempferol, epimanoyl oxide, ferulic acid, p-coumaric acid, 4-hydroxybenzoic acid, α-terpineol, glyceryl linoleate, and 1-monolinolein (Table 1). Interestingly, among the high MolDock score constituents, only vladicort interacted with both Trp7986 and His789 amino acids, as two common amino acids involved in the interaction of testosterone with AR LBD, while the MolDock score of agnuside with the involvement of Asn705 and Leu701 was the highest (Fig.1).

Demographic characteristics and reproductive history of patients

As described in Table 2, the mean age of women were 32.36±6.64 years with initial EDSS of 1.839±1.09 and normal BMI (24.23±2.74). To evaluate the possible effect of some suspected resources of hormonal dysregulation on serum testosterone level, we described socioeconomic, underlying and reproductive factors and evaluated their possible association with serum testosterone levels but we did not find any significant association.

EDSS

Statistical analysis using chi-square test indicated a significant decrease in EDSS scores following three months of oral regimen with Vitagnus® (1.839 vs. 1.643, p=0.003) (Fig. 2A).

Serum testosterone levels

The level of testosterone was measured in the serum of RRMS patients at day 0 and day 91 from starting oral supplementary regimen of Vitagnus® tablets. The mean testosterone levels were statistically increased from 388.6(±0.16900) at baseline to 468.2ng/dL (±0.18144) at day 91 (p=0.026). Except two patients who showed decreased serum testosterone levels at day 91, the remaining participants showed increased blood testosterone levels but the mean serum levels (all cases) remained below the upper limit value in premenopausal women (Fig. 2B).

Clinical signs and MSQOL-54 functions:

As described in Table 1, 16 (57.1%) women reported menstrual irregularity at the beginning of the study but this number decreased to zero at the completion of the intervention ($p<0.001$) that means all enrolled women experienced regular (25-30 days interval) menstrual cycles using Vitagnus® supplement (n=28). Out of 28 patients, 20 women (19 married and 1 single) answered to sex related questions before and three months after intervention (Table 3). Overall sexual satisfaction (orgasm and partner satisfaction=0.005) and sexual function (sexual interest, vaginal lubrication and frequency of intercourse=0.001) were significantly improved by MSQOL-54 scoring system.

MSQOL-54 data indicated significant improvements in some other quality of life parameters following three months of treatment. Although VAC did not exhibit substantial differences in physical and cognitive functions of MS patients ($p>0.05$), it induced overall improvement in quality of life ($p=0.016$) by changing many factors as we described in details in Table 3.

Adverse drug reactions:

Out of 30 patients who met our inclusion criteria, two patients discontinued this study. The first patient stopped the pills because she experienced inflammation, erythematous rash and pruritus in her face and neck. She was vegetarian with history of allergic reactions but all signs and symptoms of drug allergy disappeared after drug withdrawal. The second women left the study because she became pregnant through uncontrolled intercourses during this study. Other than above cases, 2 patients experienced stomach disturbances and diarrhea in the first week of trial which resolved in the second week of taking Vitagnus®.

Discussion

In addition to increased risk of RRMS incidence in premenopausal women, higher rate of relapse in female patients emphasize the importance of differential and innovative supportive care methods (8). In this study, we found the value of VAC as a promising and safe supplement that improves EDSS and serum testosterone levels with concomitant clinical improvements by regulating menstrual cycles, lowering stress levels, improving vitality and emotional health and normalizing sexual life of patients. Although the clinical applicability of SEMA3A and prolactin as sex specific MS biomarkers have been already described and their contribution to MS disability and progression have been demonstrated (23), utilization of testosterone could be considered as a new therapeutic endpoint in RRMS women who are suffering from sexual and emotional discomforts, and based on our study results, VAC could be suggested as a promising candidate

for further clinical assessments in randomized clinical trials in larger sample size.

Androgen receptor (AR) is a transcription factor which activates through testosterone binding in the cytoplasm (Collongues N, 2018) and the key involvement of amino acids, which we stated as important amino acids in drug receptor interactions has been recently explained by other scientists(24) .According to the present Mol doc screening on aucubin as the standardized constitute of Vitagnus pills and its higher binding capacity to the ligand-binding domain (LBD) of AR, this theoretical part of our initial assessment was translated to significant hormonal changes and clinical improvement without recordable adverse health effects in this 3 months study. In fact, serum testosterone elevation was coincided with EDSS decrease and MSQL-54 improvement but other hormones and MS biomarkers were not considered in the present study after this pilot study; therefore, more biomarkers should be assessed in further studies.

One study on male MS patients has emphasized lower serum testosterone, DHEA, or DHEA-S levels compared to healthy age-matched control group and suggested the protective effects of testosterone through immune modulation before observing the improving role of testosterone in cognitive performance and brain atrophy by increasing gray matter size in this small cohort (9). We showed also the elevated serum testosterone levels in study groups of patients without any direct impact on their cognition performance and immune system. Additional studies in the same setting could answer these essential questions by focusing on more prognostic biomarkers of MS in longer periods compared to control group.

Androgens play different roles in women's health but androgen therapy for female sexual dysfunctions is still controversial (25,26). Age related decline in ovarian production and adrenal androgen synthesis may impair sexual function, libido, well-being, and energy even in non-MS women (27,28). We observed these general common symptoms in MS women at baseline that were controlled by VAC treatment but differential role of VAC constituents in standard pharmaceutical dosages should be determined for other hormonal factors, immune system, neural tissue and myelination to confirm these preliminary promising observations. Given virtual affinities of VAC components to AR LBD, common expectable androgenic effects including hirsutism and acne were expectable (29) but not detected in any patients that emphasize the differential biological effects of VAC from natural androgenic hormones (30-31) and clarify whether the apparent beneficial effects of VAC treatment could result from additional receptor interactions.

Conclusion

VAC treatment by predictable androgenic effects and AR LBD affinity caused significant improvement in quality of life and EDSS score of premenopausal RRMS patients. We described these impressive effects by

elevated serum testosterone levels but the manipulating role of VAC constituents in other sex hormones and neurotransmitters should be followed by later studies. According to our results, we recommend the use of Vitagnus® tablets as a suitable, economical, simple and safe way to relieve menstrual irregularity and EDSS in premenopausal MS patients which improves sexual dysfunctions in MS women who are suffering from persistent sexual complications. Long-term efficacy and safety and its cost-effectiveness for volunteering patients with tendency to herbal medicine as well as other clinical effects should be followed in next studies based on extensive neuroprotective, anti-inflammatory and antioxidant roles of VAC constituents due to its various compounds.

References

1. Li R, Sun X, Shu Y, Mao Z, Xiao L, Qiu W, Lu Z, Hu X. Sex differences in outcomes of disease-modifying treatments for multiple sclerosis: A systematic review. Mult Scler Relat Disord.; 2017,12, 23-28.

2. Marrie RA, Cohen J, Stuve O, Trojano M, Sørensen PS, Reingold S, Cutter G, Reider N. A systematic review of the incidence and prevalence of comorbidity in multiple sclerosis: overview. Mult Scler.; 2016,21(3), 263-81.

3. CE. Markowitz. Multiple sclerosis update. Am J Manag Care.; 2013, 19(16),294-300.

4. Olsson T Barcellos LF, Alfredsson L. Interactions between genetic, lifestyle and environmental risk factors for multiple sclerosis . Nat Rev Neurol.; 2017,13(1) , 25-36.

5-Leray E Moreau T, Fromont A, Edan G. Epidemiology of multiple sclerosis .Rev Neurol (Paris); 2016,172(1),3-13.

6- Shen Q Lu H, Xie D, Wang H, Zhao Q, Xu Y. Association between suicide and multiple sclerosis: An updated meta-analysis .Mult Scler Relat Disord.;2019,34, 83-90.

7- Dehghan-Nayeri N Khakbazan Z, Ghafoori F. Life behind the mask": Sexual life of Iranian women with multiple sclerosis . Disabil Health J.; 2018, 11(2),274-280.

8-Dunn SE Lee H, Pavri FR, Zhang MA. Sex-Based Differences in Multiple Sclerosis (Part I): Biology of Disease Incidence . Curr Top Behav Neurosci. ;2015, 26, 29-56.

9- Gubbels Bupp MR Jorgensen TN. Androgen-Induced Immunosuppression .Front Immunol.;2018,9,794.

10- Trigunaite A Dimo J, Jørgensen TN. Suppressive effects of androgens on the immune system.Cell Immunol.;2015: 294(2) , 87-94.

11-Tavakol S Shakibapour S, Bidgoli SA. The Level of Testosterone, Vitamin D, and Irregular Menstruation More Important than Omega-3 in Non-Symptomatic Women Will Define the Fate of Multiple Scleroses in Future [. Mol Neurobiol.; 2018 : 55(1),462-469.

12-Valvano AK Rollock MJD, Hudson WH, Goodworth MR, Lopez E, Stepleman L. Sexual communication, sexual satisfaction, and relationship quality in people with multiple sclerosis . Rehabil Psychol.; 2018: 63(2) ,267-275.

13- Cerqueira RO Frey BN, Leclerc E, Brietzke E. Vitex agnus castus for

premenstrual syndrome and premenstrual dysphoric disorder: a systematic review . Arch Womens Ment Health. ;2017, 20(6), 713-719.
14- Nasri S Oryan S, Rohani AH, Amin GR. The effects of Vitex agnus castus extract and its interaction with dopaminergic system on LH and testosterone in male mice .Pak J Biol Sci. ;2007 : 10(14), 2300-7.
15- Sehmisch S Boeckhoff J, Wille J, Seidlova-Wuttke D, Rack T, Tezval M, Wuttke W, Stuermer KM, Stuermer EK. Vitex agnus castus as prophylaxis for osteopenia after orchidectomy in rats compared with estradiol and testosterone supplementation .Phytother Res.;2009 : 23(6), 851-8.
16- Meier B Berger D, Hoberg E, Sticher O, Schaffner W. Pharmacological activities of Vitex agnus-castus extracts in vitro.Phytomedicine.;2000:7(5),373-81.
17-Webster DE Lu J, Chen SN, Farnsworth NR, Wang ZJ. Activation of the mu-opiate receptor by Vitex agnus-castus methanol extracts: implication for its use in PMS .J Ethnopharmacol. ;2006; 106(2),216-21.
18- Singh SP Deb CR, Ahmed SU, Saratchandra Y, Konwar BK. Molecular docking simulation analysis of the interaction of dietary flavonols with heat shock protein 90 . J Biomed Res. ;2015,30.
19- Zhou W Duan M, Fu W, Pang J, Tang Q, Sun H, Xu L, Chang S, Li D, Hou T. Discovery of Novel Androgen Receptor Ligands by Structure-based Virtual Screening and Bioassays . Genomics Proteomics Bioinformatics.;2018 : 16(6), 416-427.
20- Hajimehdipoor H Shekarchi M, Piralihamedani M, Abedi Z, Zahedi H, Shekarchi M, Gohari AR. A Validated HPTLC-Densitometric Method for Assay of Aucubin in Vitexagnus-castusL .Iran J Pharm Res.;2011: 10(4), 705-10.
21- McNicholas N Hutchinson M, McGuigan C, Chataway J. 2017 McDonald diagnostic criteria: A review of the evidence .Mult Scler Relat Disord.;2018: 24, 48-54.
22-JF. Kurtzke Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS) . Neurology;1983, 33(11),1444-52.
23- Jacob S Al-Kandari A, Alroughani R, Al-Temaimi R. Assessment of plasma biomarkers for their association with Multiple Sclerosis progression .J Neuroimmunol.;2017:305, 5-8.
24- Al-Imari L Wolfman WL. The safety of testosterone therapy in women . J Obstet Gynaecol Can; 2012;34(9) , 859-865.
25- Pluchino N Carmignani A, Cubeddu A, Santoro A, Cela V, Errasti T. Androgen therapy in women: for whom and when .Arch Gynecol Obstet. ;2013:288(4),731-7.
26-Kalantaridou SN Calis KA. Testosterone therapy in premenopausal women.Semin Reprod Med; 2006:24(2),106-14.
27-Collongues N Patte-Mensah C, De Seze J, Mensah-Nyagan AG, Derfuss T. Testosterone and estrogen in multiple sclerosis: from pathophysiology to therapeutics . Expert Rev Neurother. ;2018:18(6) , 515-522.
28-Golden LC Voskuhl R. The importance of studying sex differences in disease: The example of multiple sclerosis .J Neurosci Res; 2017 J:95(1-2) , 23017.
29- Feinstein A Pavisian B. Multiple sclerosis and suicide . Mult Scler. ; 2017:23(7),923-927.

30- Wang C Liu Y, Cao JM. G protein-coupled receptors: extranuclear mediators for the non-genomic actions of steroids. Int J Mol Sci.; 2014: 15(9), 15412-25.
31- Li R Sun X, Shu Y, Mao Z, Xiao L, Qiu W, Lu Z, Hu X. Sex differences in outcomes of disease-modifying treatments for multiple sclerosis: A systematic review .Mult Scler Relat Disord.; 2017: 12, 23-28.