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Use of a Natural Compound Made of *Ecklonia bicyclis* Seaweed, *Tribulus terrestris* and Water-Soluble Chitosan Oligosaccharide, in Male Sexual Asthenia with Mild or Mild-Moderate Erectile Dysfunction and Serum Testosterone Levels at the Lower Limit of Normal

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Abstract

Objectives: to evaluate the effectiveness of a natural compound made of *Ecklonia bicyclis* Seaweed, *Tribulus terrestris* and water-soluble chitosan oligosaccharide, in the male sexual asthenia with mild or mild-moderate erectile dysfunction and serum testosterone levels between 280 and 350 ng/dl. **Materials and Methods:** 84 male patients affected by reduced libido and serum testosterone levels at the lower limit of normal, were recruited. We have separated patients in three different age groups: group A (18 - 45 years), group B (45 - 59 years), group C (>60 years). All subjects answered the International index of erectile function questionnaire (IIEF-5) and underwent determination of serum total testosterone before and after 30 days of treatment. **Results:** Before treatment, the group A showed mean (± standard deviation) total testosterone 321.9 ± 19.2 ng/dl and mean IIEF-5 18.6 ± 1.97, in the group B it was 318.5 ± 18.1 ng/dl and 16.3 ± 2.66, and finally in the group C it was 305.4 ± 13.1 ng/dl and 14.2 ± 1.95 respectively. After treatment mean total testosterone and mean IIEF-5 were respectively: group A (448 ± 111.46 ng/dl and 21.84 ± 3.41); group B (453.8 ± 105.23 ng/dl and 20.4 ± 3.81); group C (385.8 ± 87.29 ng/dl and 16.7 ± 3.84). **Conclusions:** The treatment with *Ecklonia bicyclis*, *Tribulus terrestris* and water-soluble chitosan oligosaccharide might represent a safe and effective option on the improvement of libido and erectile function in man with testosterone level at the lower limit of normal.

Keywords

Sexual Dysfunction, Sexual Asthenia, Testosterone, *Ecklonia bicyclis* Seaweed, *Tribulus terrestris*, Herbal Medicine

1. Introduction

Several studies have tried to evaluate the possible relationship between serum levels of testosterone and sexual function [1] [2]. Some meta analyses have shown that for some aspects of sexuality like libido, erectile function, orgasmic function and overall sexual satisfaction, it does not exist a close correlation with androgens serum levels, while they could be related to more complex mechanisms, which also embrace the psycho-social sphere [3]. The International Society for Study of the Aging Male (ISSAM) defines the Androgens Deficiency Syndrome (ADS) as "a biochemical syndrome associated with advancing age and characterized by a deficiency in serum androgen levels with or without a decreased genomic sensitivity to androgens"; this condition is known as late-onset hypogonadism (LOH).

The true prevalence of ADS in adult men is unknown as a result of inconsistent definitions used in literature. Population-based studies suggest the prevalence to be between 2.1% and 38.7% [4] [5]. The prevalence of AD in men suffering from systemic diseases is significantly higher than those not evincing these diseases. Physicians caring for these patients need to be aware of the increased prevalence and need to offer appropriate screening [6]. Symptoms related to ADS are very heterogeneous and they include erectile dysfunction (ED), decreased libido, nervousness, asthenia, insomnia, anxiety, depression, cognitive decline and several conditions that may reduce quality of life and may cause an increase in mortality, due to an increase in cardiovascular risk, endothelial dysfunction, metabolic syndrome, osteoporosis etc. [7] [8] [9]

It is estimated that the serum levels of testosterone decrease at a rate of 1% - 2% [10] per year. However, unlike female menopause, which is a universal process associated with aging, the exact rate of decline and presenting symptoms are highly variable in men. At the same time, biochemical measurements among different assays also produce non-uniform reference ranges because of assay sensitivity variation, making diagnosis difficult [11]. Heightened awareness of AD has led to the development of many treatment options for LOH. Literature is limited regarding the long-term outcomes of LOH. Despite the wide recognition and adaptation to intervention, debate is ongoing regarding the benefits and the risks associated with treatment [12].

Furthermore, the ED has different etiologies in addition to ADS and despite the good results, with the introduction of PDE-5 inhibitors, physicians have still some difficulties to manage both the subjects who do not respond to PDE-5 inhibitors (approximately 30%) and those in which serum levels of testosterone are at low normal limits but not enough to merit a hormonal replacement therapy [13] [14]. Meta-analyses of randomized, placebo-controlled trials have showed that testosterone therapy in patients with

borderline biochemical AD was associated with minimal improvement in erectile function (95% confidence interval [CI] 0.03 to 0.65), non-significant effect on libido (95% CI 0.01 to 0.83), and no effect on overall sexual satisfaction [15].

Therefore facing with these problems as well as the general trend of both physicians and patients to choose treatment options with limited side effects, we are increasingly moving towards phytotherapic alternatives that could overcome this therapeutic gap.

In the oriental medicine, *Tribulus terrestris* has different therapeutic properties such as androgen-mimetic, aphrodisiac, anti-inflammatory, anti-hypertensive and spermatogenic effects. These properties seem to be ascribed to the presence of saponins including protodioscin that for its steroidal structure binds the androgen receptors and causes an increase in endogenous serum levels of testosterone, dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS) and hormone luteinizing (LH). In addition, *Tribulus terrestris* has a pro-erectile function due to its ability to determine the release of nitric oxide in the corpora cavernosa. These effects have been confirmed by several studies in rabbits and primates, while in humans we still have conflicting data [16]-[23]. In the last years Ecklonia seaweed, has more potential health beneficial applications in several fields, for various beneficial biological activities such as anti-age, anti-hypertensive, anti-diabetic, anti-cancer and anti-fibrotic. Ecklonia is a species of edible brown seaweeds that includes three types: *Ecklonia cava*, *E. stolonifera* and *E. bicyclis*. Its application in sexual dysfunction is mainly due to its scavenger activity against reactive oxygen species (ROS) responsible for various local and general structural alterations [24] [25] [26] [27]. Nitric oxide is an important factor involved in the mechanisms of erection. There are several natural substances capable of increasing the endogenous production of nitric oxide, one of this is the chitosan oligosaccharide utilized in this study [25].

Our study was conducted using a combination of *Tribulus terrestris*, *Ecklonia bicyclis* seaweed and a water-soluble chitosan oligosaccharide to evaluate the synergistic effects of this combination on sexual asthenia in ED-treatment naive subjects with mild or mild-moderate ED assessed by an IIEF-5 questionnaire and serum testosterone levels at the lower limit of normal, precisely between 280 and 350 ng/ml.

2. Methods

From November 2015 to May 2016, we prospectively enrolled 84 males presenting with mild and mild-moderate ED ($12 < \text{IIEF-5} < 21$) and serum testosterone levels between 280 ng/dl and 350 ng/dl.

Inclusion criteria:

- 18 years of age or older;
- Erectile dysfunction treatment-naive;
- Serum testosterone levels between 280 ng/dl and 350 ng/dl;
- Serum LH, FSH, E2, PRL normal levels;
- $12 \leq \text{IIEF-5} \leq 21$.

Exclusion criteria were as follows:

Dyslipidemia, uncontrolled diabetes mellitus, uncontrolled hypertension, previous pelvic radiotherapy, previous pelvic surgery, use of phosphodiesterase-5 inhibitor, patients using anabolic steroids, Peyronie's disease, hormonal disorders and depression (Table 1).

All patients included in the trial gave written informed consent before entering the study, which was conducted in accordance with the Declaration of Helsinki. All subjects were evaluated with physical examination of genitals, detailed history about their sexual relationship, morning erections and level of libido. Each patient received a tablet orally, consisted of 300 mg of *Ecklonia bicyclis* seaweed, 450 mg of *Tribulus terrestris* and 250 mg of Chitosan oligosaccharide (D-Glucosamine and N-Acetyl-D-Glucosamine) once a day for 30 days. At the end of the treatment, they were instructed to dose serum total testosterone level, and again to respond to the IIEF-5 questionnaire.

All patients were divided in three groups according to their age: Group A 18 - 45 years, Group B 46 - 59 years, Group C > 60 years old.

Statistical Analysis Used

Statistical significance was determined by SPSS, version 20.0. Analysis of continuous variables was assessed by paired t-test. The Spearman coefficient of rank correlation was also used to test the correlation between the level of total testosterone and IIEF-5 before and after treatment. Data were reported as means ± standard deviation (SD) and nominal p values were presented. For all statistical analysis, significance was considered as $p < 0.05$.

3. Results

Among 84 patients enrolled in our Urology Department, seventy-eight patients (92.86%) completed the cycle of treatment and were considered for outcome analysis. Four (4.7%) patients stopped treatment. We were unable to trace two patients (2.3%), who were then considered not to have completed the treatment. Mean age was 45.5 years with a SD of 13.57. Group A (18 - 45 years old) was composed by 37 subjects with a mean age of 32.8 ± 5.66; Group B (46 - 59 years old) was composed by 30 subjects

Table 1. Inclusion and exclusion criteria for the study assessing the efficacy of treatment with a combination of *Ecklonia bicyclis*, *Tribulus terrestris* and Water soluble chitosan oligosaccharide in men with low libido and serum testosterone level at the lower limit of normal.

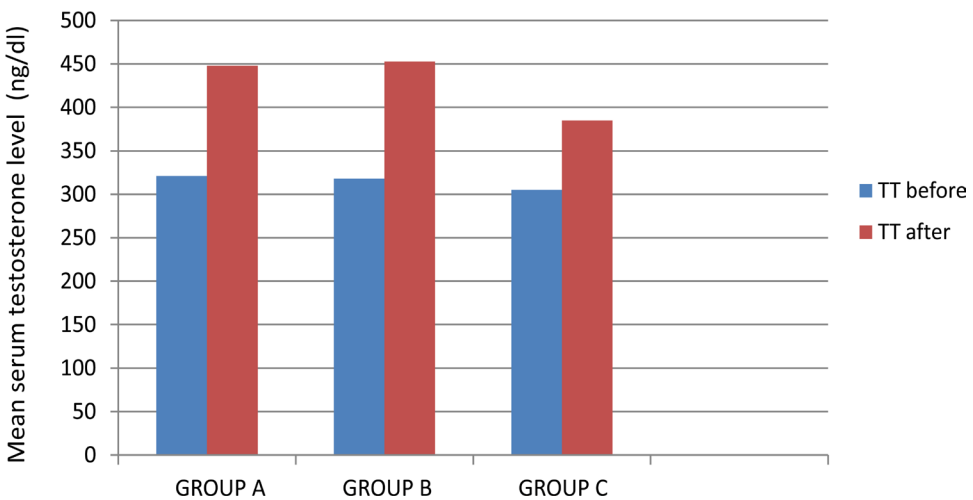
Inclusion criteria	Exclusion criteria
18 years of age or older	Uncontrolled diabetes mellitus
Erectile dysfunction treatment-naive	Dyslipidemia
Serum testosterone levels between 280 and 350 ng/dl	Peyronie's disease
IIEF-5 between 12 and 21	Previous pelvic surgery
Normal levels of LH, FSH, E2 and PRL	Depression
	Hormonal disorders

with a mean age of 53.8 ± 3.48 and Group C (>60 years old) was composed by 11 subjects with a mean age of 65.7 ± 4.38.

Pre-treatment mean (±standard deviation) total testosterone serum levels were 318.27 ng/dl ± 18.68 with a mean IIEF-5 of 17.10 ± 2.66. The mean values for each group are shown in the table below. Post-treatment mean total testosterone serum levels were 441.67 ng/dl ± 107.21, with a mean IIEF-5 of 20.57 ± 3.96. The differences of both testosterone and IIEF-5 between the pre and post-treatment values are all statistically significant (Figure 1 & Figure 2). Moreover our study using Spearman's correlation significance two-tailed test, has shown statistically significant correlation between the serum level of total testosterone and IIEF-5 before and after treatment: the pre-treatment index was 0.20 in Group C (>60 years) 0.66 in Group B (46 - 59 years) and 0.27 in Group A (18 - 45 years), conversely the post-treatment index was 0.73, 0.58 and 0.65 for all aforesaid groups (Table 2).

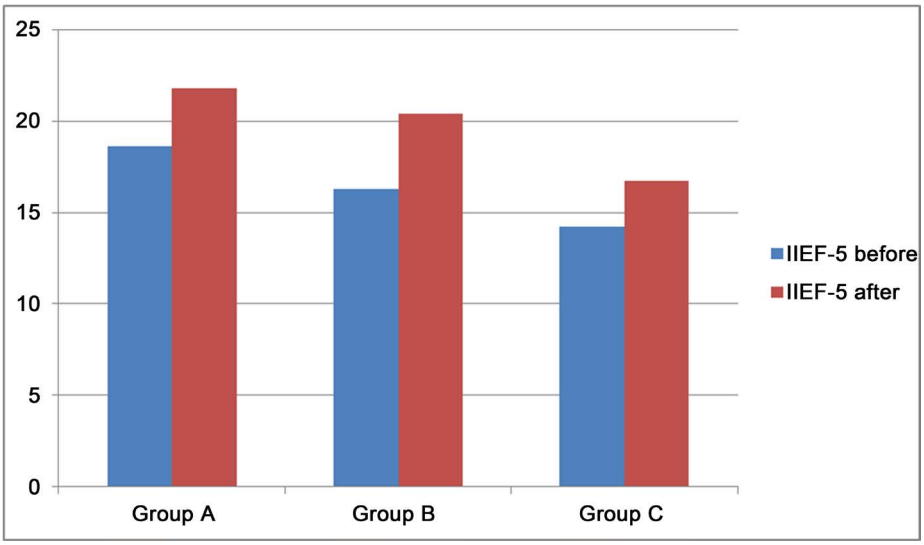
4. Discussion

Despite the well known efficacy of oral PDE-5 inhibitors therapy for ED as well as the testosterone supplement in conditions of hypogonadism, the management of those patients who experience ED associated with low libido that do not respond to PDE-5 inhibitors showing normal hormone levels or in any case at the lower limit of normal, still remains complicated. In fact, we do not know yet the exact biochemical threshold serum testosterone concentration below which symptoms of AD and adverse outcomes occur. The testosterone replacement therapy is reserved for patients with severe declines in androgenic serum levels, demonstrating a decline in muscle mass and strength, a reduction of bone mineral density, and a decrease in sexual functions. Fur-



TT: Total Testosterone; SD: Standard Deviation

Figure 1. Difference of mean serum testosterone levels before and after 30 days treatment period. Data are mean ± SD. Before treatment, the group A showed total testosterone 321.9 ± 19.2 ng/dl, the group B 318.5 ± 18.1 ng/dl, the group C 305.4 ± 13.1 ng/dl. After treatment mean total testosterone levels were: group A (448 ± 111.46 ng/dl); group B (453.8 ± 105.23 ng/dl); group C (385.8 ± 87.29 ng/dl).



IIEF-5: International Index of Erectile Function; SD: Standard Deviation

Figure 2. Difference between mean IIEF-5 before and after 30 days treatment period. Data are mean ± SD. Before treatment, the group A showed IIEF-5 18.6 ± 1.97, the group B 16.3 ± 2.66 and the group C 14.2 ± 1.95. After treatment IIEF-5 were: group A (21.84 ± 3.41); group B (20.4 ± 3.81); group C (16.7 ± 3.84).

Table 2. Mean changes from baseline to 30 days of treatment.

Group age	Mean age ± SD	Number	TT before	TT after	*p*-value	IIEF-5 before	IIEF-5 after	*p*-value
A: 18 - 45	32.8 ± 5.66	37	321.9 ± 19.2	448 ± 111.46	<0.05	18.6 ± 1.97	21.84 ± 3.41	<0.05
B: 46 - 59	53.8 ± 3.48	30	318.5 ± 18.14	453.8 ± 105.23	<0.05	16.3 ± 2.66	20.4 ± 3.81	<0.05
C: >60	65.7 ± 4.38	11	305.4 ± 13.16	385.8 ± 87. 29	<0.05	14.2 ± 1.95	16.7 ± 3.84	<0.05
Total: A + B + C	45.5 ±13.57	78	318.3 ± 18.67	441.7 ± 107.21	<0.05	17.10 ± 2.66	20.57 ± 3.96	<0.05

SD: Standard deviation; TT: Total Testosterone; IIEF-5: International Index of Erectile Function.

thermore, as well as PDE- 5 inhibitors therapy, testosterone treatment is not free of side effects that sometimes restrict the range of patients who can benefit from, especially in those with high risk of cancer or with several associated comorbidities. In this scenario the use of phytotherapic products plays an important role. *Tribulus terrestris* (TT) is a plant used in the Eastern tradition, belonging to the Zygophyllaceae family that includes more than twenty species and is present in many European countries. There are several uses of this compound: astringent, anti-hypertensive, palliative, tonic, diuretic and urinary disinfectant, fertilizing. The role of this phytocompound on erectile function is related to its aphrodisiac and androgen-mimetic effect carried by the saponins present in it and especially thanks to the Protodioscin (5,6- diidroprotodioscin) that in addition to its activity on the androgen receptors, it would seem to be able to convert testosterone into its active compound, dihydrotestosterone, which occurs by the action of 5-alpha reductase [16].

Miliasius et al. in a study on athletes, assert that the improvement of erectile function

may be due to the conversion of testosterone in DHEA [26]. *Gauthman et al.* have shown that in mice, rabbits and monkeys, the oral administration of *Tribulus terrestris* was able to increase the serum levels of both testosterone and dihydrotestosteroneand and intracavernous pressure thus justifying the pro-erectile effect of Protodioscin [21]. The vasodilatory effect of Protodioscin had already been demonstrated by a Chinese study conducted in 406 patients with angina pectoris [27]. However, recent studies conducted on rats do not seem to attribute an intrinsic hormonal activity to *Tribulus terrestris* [28]. *Do et al.* justify the effectiveness of *Tribulus terrestris* on erectile function through its ability to activate the pathways of NO/NO synthase leading to an effect on the endothelium of the corpora cavernosa [29]. Nevertheless, the data reported on human studies concerning the effects of Tribulus on testosterone serum levels are inconclusive and often contradictory. *C. A. Santos et al.* claim that there is no difference between Tribulus and placebo neither on improving ED nor on the increase in serum testosterone levels [30]. On the other hand, *Roaiah et al.* claim that Tribulus is a good therapeutic option for patients suffering from ED with PADAM (partial androgen deficiency of the ageing male) as it is capable to raise both the IIEF-5 score and serum testosterone levels [31]. A recent review also concludes that Tribulus has a positive effect on libido and erectile function but the data regarding its androgenic properties are still not conclusive [32]. Our study shows that the synergistic action of the three compounds, *Ecklonia bicyclis*, oligosaccharide water-soluble chitosan and *Tribulus terrestris*, is able to lead to an improvement of erectile function by acting at multiple levels, improving both serum testosterone levels and libido [23] [24]. These effects may be justified, in addition to the action of Tribulus, which in the compound used by us is titrated at 90% of saponins, also by the action of the polymers of D-glucosamine and N-acetyl-glucosamine present in the oligosaccharide chitosan. These amines contribute to increase the NO levels in the smooth muscle cells of the corpora cavernosa through induction of endothelial NO synthase. In addition, the powerful antioxidant action of Ecklonia seaweed for the presence of phlorotannins and polyphenols represents a further protection mechanism against the cellular ageing caused by reactive oxygen species (ROS). As a matter of fact in the rough fraction of seaweed there are high concentrations of phlorotannins like dieckol, phlorofucofuroeckol-A (PFE-A) and bieckol that have a scavenger effect 10 to 100 times more powerful than the catechins present in green tea. Antioxidant activity was detected in each alga: *E. bicyclis* water extract (91%), followed by *E. stolonifera* (90%) and *E. cava* (74%) [33]. Additionally, the action on the lipoxygenase, on the TNF-alpha, on cyclooxygenase-2 and on the interferon gamma, coupled with its increased half-life compared to common polyphenols, makes *E. cklonia* a great anti-fibrotic and anti-oxidant aid [34] [35] [36]. Major mechanisms of endothelial dysfunction include the down-regulation of endothelial nitric oxide synthase levels, differential expression of vascular endothelial growth factor, inflammatory pathways and oxidative stress. Endothelial Dysfunction tends to be the initial event in macrovascular and microvascular complications. Numerous strategies have been developed to protect endothelial cells against various stimuli, in which the role of poly-

phenolic compounds in modulating the different pathways and thus maintaining vascular homeostasis has been proven to be beneficial. Different experimental observations support the existence of a molecular/biochemical link between vasodilation, nitric oxide (NO) production and angiogenesis. Nitric oxide contributes to the pro-survival/pro-angiogenic program of capillary endothelium by triggering cell growth and differentiation via endothelial-constitutive NO synthase (eNOS) activation and cyclic GMP (cGMP) dependent gene transcription. ROS forming oxidant peroxynitrites (ONOO-) enhance endothelial dysfunction by direct uncoupling and inactivating the eNOS [37]. Re-establishment of a balanced nitric oxide production with an anti-ROS activity causes a reduction of cell damage. It has been reported that vasculogenic ED patients have elevated levels of angiotensin II for the whole duration of the erectile process. The demonstrated action of Ecklonia seaweed on ACE resulting in vasodilation plays an important role in inducing successful erectile function. As discussed, phlorotanins have strong anti-oxidant, anti-fibrotic and anti-inflammatory effects. Together with Ecklonia's ACE inhibitory activity, these effects, with a long-term oral administration, may all contribute to support vascular system, including the penile arteries [38]. All these mechanisms result in long-term protective effects on the corpora cavernosa making the combination of these compounds a reasonable preventive solution.

5. Conclusion

In conclusion, from this study it emerges that the use of the phytotherapeutic complex consisting of Tribulus, *Ecklonia bicyclis* and water-soluble oligosaccharide chitosan, is able to improve sexual function through both an increase of the serum levels of testosterone and an increase in libido in subjects having mild or mild-moderate ED with serum testosterone levels at the lower limit of normal. This benefit is expressed for all age groups analyzed (18 - >60 years old). It is a good therapeutic option, with minimal or no side effects, for both treatment of naïve patients and for those not susceptible to drug treatments.

Limitations

Limitations of this study are the absence of a placebo group of comparison and randomization. Moreover large sample size is necessary to evaluate if there is an age group that could benefit from this treatment more than the others.

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