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ORIGINAL PAPER

A new original nutraceutical formulation ameliorates the effect of Tadalafil on clinical score and cGMP accumulation

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Summary *Objective: To assess the efficacy of the combination of Tadalafil 5 mg and nutritional supplements composed by Panax ginseng, Moringa Oleifera and Rutin on erectile function in men with mild and moderate vasculogenic ED.*

Methods: we prospectively enrolled 86 patients divided into two groups A (45), B (33) in this multicenter randomized, double-blind, placebo-controlled trial . Drop out was 8 patients (3 patients in group A and 5 in Group B). At screening visit patients underwent clinical examination, blood test (hormonal and metabolic profile) and filled out the IIEF-5 questionnaire and the SEP-2, SEP-3. Patients were randomized by a computer-generated list to receive either Tadalafil 5 mg once daily plus nutritional supplement once daily (group A) or Tadalafil 5 mg plus placebo with the same administration schedule (group B) for 3 months. Blood samples, IIEF-5, SEP-2 and SEP-3 have been collected again after 3 months. cGMP was measured in platelets of 38 patients at baseline and after one months.

Results: Mean age was 59.98 ± 6.90 (range 38-69), mean IIEF-5 score at baseline was 13.59 ± 3.90. After three months of treatment, IIEF-5 score significantly improved in both groups compared to baseline (13.18 ± 3.75 vs 20.48 ± 2.24, p < 0.0001; 14.15 ± 4.09 vs 19.06 ± 4.36, p < 0.0001, in group A and group B respectively). Patients treated with Tadalafil plus nutritional supplement showed a significantly higher increase in IIEF-5 score compared to those who received placebo (7.27 ± 2.20 and 4.9 ± 2.79, respectively; p < 0.0001;). No hormonal differences and metabolic effects were found. According cGMP result, nutritional supplements ameliorates and extends the activity of the chronic treatment.

Conclusions: IIEF-5 significant increase in group B, can be ascribed to the nutritional supplement properties and antioxidant effects of moringa oleifera, ginseng and rutin and this can enhance the endothelial NO and cGMP production.

KEY WORDS: *Erectile dysfunction; PDE5; Dietary supplement; Phosphodiesterase: Natural health product.*

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INTRODUCTION

Erectile dysfunction (ED) has been identified as the most common sexual problem, with high prevalence and incidence worldwide, that affects the *quality of life* (QoL) of patients and their partner's. It is estimated that about 322 million men would suffer from ED global by the year 2025 (1). ED primarily affects men older than 40 years of age. ED prevalence ranges between 1-10% in men younger than 40 years (*International Consultation Committee for Sexual Medicine*). The prevalence increases with age in a range from 2% to 9% in men between the ages of 40 and 49 years, 20-40% in men aged 60-69 years and > 50% in men older than 70 years (2). To the ED onset contributes several environmental and lifestyle risk factors such as diabetes mellitus, hypertension, hyperlipidaemia, obesity, metabolic syndrome, depression, smoking and limited or absence of physical exercise (3-7).

Penile erection is a complex of events controlled by vascular, hormonal and neuronal systems (8). For what concerns the vascular component the endothelium plays a major role through the *nitric oxide* (NO) pathway. Indeed, the activation of the NO pathway causes relaxation of smooth muscle in the penile corpus cavernous, leading to increased inflow of blood (9). NO is synthesized within the endothelium starting by *endothelial nitric oxide synthase* (eNOS) and activates the *soluble guanylyl cyclase* (sGC) that leads to the formation of *cyclic guanosine monophosphate* (cGMP). The cGMP levels are tightly controlled by *phosphodiesterases* (PDEs) (10). Nowadays, medical interventions for ED management include oral drugs, intrapenile therapies (intra-urethral suppositories and intracavernous injections) and penile prosthesis implantation (11). The most widely used therapeutic approach relies on the use of *phosphodiesterase type 5* (PDE5) inhibitors (12-13). The PDE5 response rate is about 70 %.and it is significantly lower in difficult-to-treat subpopulations (14). Many studies have also shown that the dropout rate with PDE5 inhibitors therapy is still more than 50% after one year (15). Emerging shreds of evidence propose an increasing role of herbal-based dietary supplements and nutraceuticals in the management of ED, for their anti-oxidant, anti-inflammatory and anti-proliferative properties.(16).

No conflict of interest declared.

The study aims to evaluate the efficacy of the combination of Tadalafil 5 mg and nutritional supplements composed by *Panax ginseng, Moringa oleifera* and *rutin* on erectile function in men with mild and moderate vasculogenic ED. In order to address this issue, we applied two different approaches: i) the assessment of *Index of Erectile Function* (IIEF) and *Sexual Encounter Profile* (SEP) that represent primary endpoints in clinical studies on ED (17); ii) the measurement of platelet cGMP content that represents biomarker of PDE5 activity (18-19).

Methods

The study consisted of two different phases: a clinical one and an experimental *ex vivo* one.

In a multicenter randomized, double-blind, placebo-controlled trial we enrolled consecutive patients with vasculogenic ED attending *Urology Clinic of University of Naples "Federico II"*, *University of Campania "Luigi Vanvitelli"* and *Interdepartmental Research Center for Sexual Medicine* (CIRMS), *University of Naples Federico II*. Inclusion criteria were: patient age between 18 and 69 years; mild to moderate ED for at least 6 months with a short form of *International Index of Erectile Function score* (IIEF-5) > 7 and < 22; hypertension treated with ACE inhibitors, beta-blockers or calcium antagonists and/or type 2 diabetes treated with oral hypoglycemic agents; the patient has been in a stable sexual relationship for > 3 months. Patients have had to be naïve for PDE5-inhibitors treatment. Exclusion criteria were: patients who had severe ED (IIEF-5 score < 8), ED due to endocrine disorders, premature ejaculation, previous pelvic surgery, Peyronie's disease, liver or renal failure, history of myocardial infarction, cardiovascular disease, stroke, unstable angina and heart failure within the previous 6 months, intake of nitrates, diabetes mellitus on insulin therapy, dyslipidemia under drug treatment, spinal cord injuries.

At screening visit patients underwent clinical examination, blood test (hormonal and metabolic profile) and filled out the IIEF-5 questionnaire and the SEP (SEP-2: «*Were you able to insert your penis into your partner's vagina?*» and SEP-3 «*Did your erection last long enough for you to have sexual intercourse?*»). Patients who met inclusion criteria were randomized by a computer-generated list to receive either Tadalafil 5 mg once daily plus nutritional supplement once daily (group A) or Tadalafil 5 mg plus placebo with the same administration schedule (group B) for 3 months. Blood samples, IIEF-5, SEP-2 and SEP-3 have been collected again after 3 months. All *adverse events* (AEs) occurred during the study period were recorded.

The study was carried out in accordance with the Declaration of Helsinki and GCP. All patients provided written informed consent. The protocol was approved by the ethical committee of *Federico II University of Napoli*.

Nutritional supplement composition

The nutritional supplement used for the study resulted from a combination of *Panax ginseng* (500 mg), *Moringa oleifera* (200 mg) and *rutin* (50 mg). The three components were assembled in a three-layer tablet that allows different timing for the release of active ingredients.

Human washed platelets

Blood samples were collected from additional patients who met inclusion and exclusion criteria described above. These subjects received Tadalafil 5 mg/daily plus nutritional supplement 1 cpr/daily for one month.

The blood samples were collected before (baseline) and after treatment.

Human washed platelets were obtained by blood (20 ml) samples collected by venipuncture. Each sample was mixed with trisodium citrate (3.8% w/v 1:10 ratio) and then centrifuged at 150 x g for 10 min to obtain *platelet-rich plasma* (PRP) as a supernatant. Washed platelets were prepared as previously described (18, 19). PRP was centrifuged at 800 × g for 12 min after the addition of 1/10 volume ACD solution (85 mM Na3-citrate, 11 mM d-glucose, 71 mM citric acid, pH 4.4). The pellet was resuspended in Ca+ 2/Mg+ 2-free HEPES-Tyrode buffer (134 mM NaCl, 12 mM $NaHCO_3$, 2.9 mM KCl, 0.36 mM Na_2HPO_4, 5 mM HEPES, 5 mM glucose, 0.5% (w/v) bovine serum albumin, pH 7.4) and adjusted to 5 × 105 platelets/µl. The platelet number was determined by using a cell counter (*AcT diff 2, Instrument Laboratory, Milan, Italy*).

cGMP measurement

Human washed platelets (5 × 105 platelets/µl) were incubated at 37 °C with vehicle or diethylamine NONOate (*DEA-NONOate, Alexis; Vinci Biochem, Vinci, Italy*), a stable donor of NO at the concentration of 10 µM or 100 µM. The reaction was stopped after 30 minutes in liquid nitrogen. DEA-NONOate spontaneously dissociates in a pH-dependent, first-order process with a half-life of 2 min at 37 °C, pH 7.4, to liberate 1.5 mol of NO per mole of parent compound (20).

Platelet suspensions were hydrolyzed with HCl 3.3 M. The lysates were centrifuged (600 × g for 10 min) and cGMP measured in supernatants as described in the manufacture's protocol of cGMP EIA Kit (*Cayman, Vinci Biochem, Vinci, Italy*) (18).

Results

A total of 86 patients were enrolled in the trial. 45 patients in group A and 33 patients in group B completed the study. Mean age was 59.98 ± 6.90 (range 38-69), mean IIEF-5 score was 13.59 ± 3.90. Table 1 showed the baseline characteristics of the two groups. No differences were noticed between groups in terms of age, baseline erectile function, comorbidities, blood tests except for total cholesterol, significantly lower in group A, and liver function tests, significantly higher in group A. After three months of treatment, IIEF-5 score significantly improved in both groups compared to baseline (13.18 ± 3.75 *vs* 20.48 ± 2.24, p < 0,0001; 14.15 ± 4.09 *vs* 19.06 ± 4.36, p < 0.0001, in group A and group B respectively, Figure 1). Patients treated with Tadalafil plus nutritional supplement showed a significantly higher increase in IIEF-5 score compared to those who received placebo (7.27 ± 2.20 and 4.9 ± 2.79, respectively; p < 0.0001; Figure 1).

A total of 28 patients (36%) completely restored their erectile function (IIEF-5 ≥ 22) and no differences were noticed between the two groups (15/45 in group A *vs*

Table 1.
Baseline characteristics of patients.

	Group A	Group B	P value
Patients. n	45	33	
MEAN AGE (range)	59.93 ± 6.75 (38-69)	60.06 ± 7.31 (43-69)	0.93
IIEF-5 MEAN (range)	13.18 ± 3.75 (8-20)	14.15 ± 4.09 (8-21)	0.28
SEP2	% (N)	%(N)	0.18
Yes	80% (36)	66.7% (22)	
No	20% (9)	33.3% (11)	
SEP3			0.60
Yes	22.2% (10)	27.3% (9)	
No	77.8% (35)	72.7% (24)	
Comorbidities	% (N)	% (N)	
Hypertension			0.17
Yes	97.8% (44)	90.9% (30)	
No	2.2% (1)	9.1% (3)	
Diabetes			
Yes	35.6% (16)	27.3% (24)	
No	64.4% (29)	72.7% (9)	0.44
Lab values			
Total testosterone	526.04 ± 128.49	481.97 ± 139.11	0.15
FSH	6.48 ± 1.96	6.16 ± 2.09	0.49
LH	6.13 ± 2.72	4.93 ± 2.72	0.06
PRL	11.43 ± 8.32	12.11 ± 4.90	0.67
Fasting blood glucose	106.80 ± 29.60	104.72 ± 27.00	0.75
Total cholesterol	186.26 ± 28.61	201.60 ± 30.67	0.02
LDL	142.68 ± 29.69	140.51 ± 25.76	0.17
AST	33.35 ± 8.28	27.06 ± 8.78	0.002
ALT	36.82 ± 9.17	30.81 ± 9.22	0.006
GGT	47.31 ± 7.55	38.03 ± 10.94	0.0001

Figure 1.
IIEF-5 score before and after three months of treatment with Tadalafil (5 mg/daily) plus nutritional supplement (1 cpr/daily), Group A, or Tadalafil (5 mg/daily) plus placebo, Group B.
Both treatments significantly increased IIEF-5 score in each group compared to baseline (Group A: 13.18 ± 3.75 vs 20.48 ± 2.24, p < 0.0001; Group B: 14.15 ± 4.09 vs 19.06 ± 4.36, p < 0.0001).
The treatment with Tadalafil plus nutritional supplement (Group A) significantly increased IEEF-5 score compared to Tadalafil plus placebo (Group B) (p < 0.0001; +7.27 ± 2.2 and 4.9 ± 2.79, respectively).
Data expressed as mean ± s.d. were analyzed by one-way ANOVA followed by Bonferroni post-test.

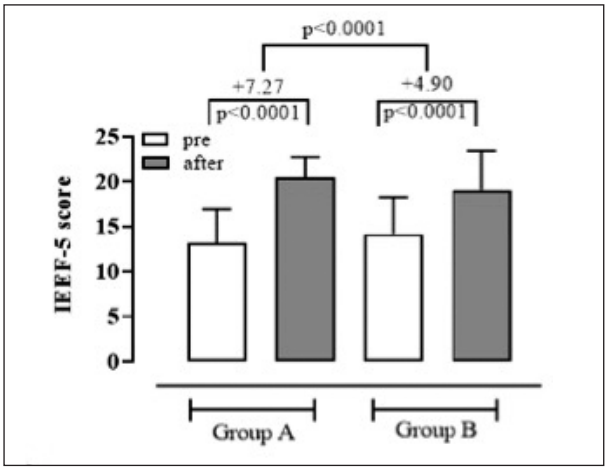


Figure 2.
SEP-2 and SEP-3 answers before and after three months of treatment with Tadalafil (5 mg/daily) plus nutritional supplement (1 cpr/daily), Group A, or Tadalafil (5 mg/daily) plus placebo, Group B. Both treatments significantly increased SEP-2 and SEP-3 in each group compared to baseline.
Data were analyzed by one-way ANOVA followed by Bonferroni post-test.
There was no significant difference between group A and group B.

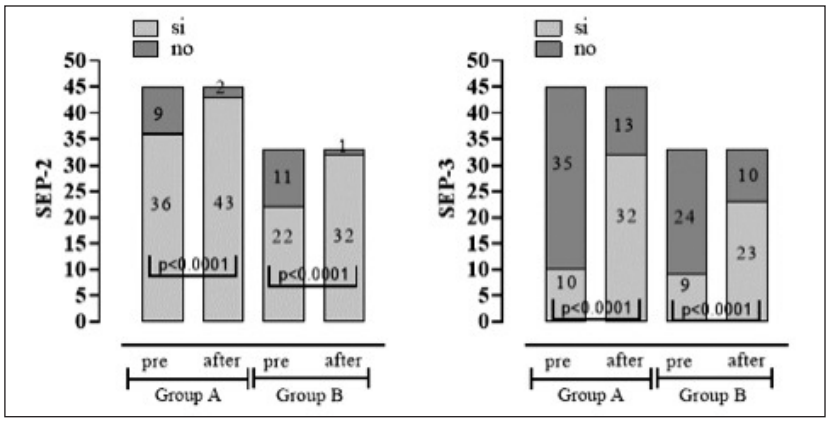


Table 2.
Hormonal levels before and after treatment.

	Group A (before)	Group A (after)	P value	Group B (before)	Group B (after)	P value
Total testosterone	526.04 ± 128.49	532.34 ± 115.73	0.82	481.97 ± 139.11	485.57 ± 122.48	0.89
FSH	6.48 ± 1.96	6.50 ± 1.77	0.96	6.16 ± 2.09	6.19 ± 2.15	0.95
LH	6.13 ± 2.72	6.38 ± 2.62	0.68	4.93 ± 2.72	5.01 ± 2.62	0.89
PRL	11.43 ± 8.32	12.61 ± 4.71	0.46	12.11 ± 4.90	12.39 ± 4.71	0.80

13/33 in group B, p = 0.58). Regarding SEP-2 and SEP-3 questions, the proportion of "*yes*" responses to SEP-2 and proportion of "*yes*" responses to SEP-3 significantly increased in both groups (p < 0.0001, Figure 2) with no differences between groups (p = 0.73 for SEP-2; p = 0.83 for SEP-3) (Figure 2). Treatment did not affect hormonal plasma levels (Table 2). No differences were noticed in metabolic profile before and after treatment in both groups. Table 3 shows adverse events related to treatment. The rate of AEs is comparable between the two groups.

Ex vivo study

The cGMP content was measured in platelets collected from 38 patients at baseline i.e. before treatment and after one month of treatment with Tadalafil 5 mg once daily plus nutritional supplement once daily.

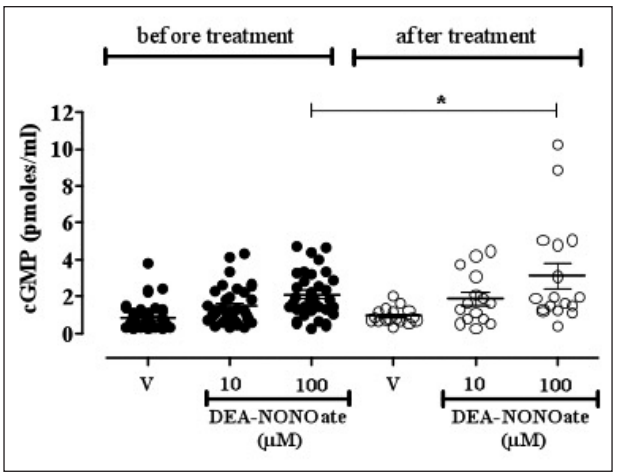
The uneven number between before and after treatment is due to the patients that have not returned because of Covid-19 pandemic.

Following one month treatment with tadalfil plus nutritional supplement significantly (*Panax ginseng*, *Moringa*

Table 3.
Adverse events.

Adverse event	Group A	Group B	P value
Headache	6	4	
Nasal congestion	1	3	
Back pain	3	1	
Dyspepsia	0	1	
Myalgia	1	1	
Cough	0	1	
Insomnia	1	0	
Flushing	6	5	
Dizziness	2	2	
Total of adverse events reported	20	18	0.38

Figure 3.
Effect of Tadalafil (5 mg/daily) plus nutritional supplement (1 cpr/daily) on platelet cGMP of ED patients.
*The levels of cGMP, expressed as pmoles/ml, were measured in 5×105/µl platelet following stimulation with vehicle (V) or diethylamine (DEA)-NONOate (10, and 100 µM). The cGMP content was evaluated before (•) or after one month of treatment (o). cGMP accumulation after treatment was significantly higher in platelets stimulated with DEA-NONOate 100 µM compared to the same concentration of DEA-NONOate before the treatment (*p < 0.05).*
Data expressed as mean ± s.e.m. were analyzed by one-way ANOVA followed by Bonferroni post-test.



oleifera and *rutin*) ($p < 0.05$) increased the cGMP content in platelets stimulated with DEA-NONOate 100 µM compared with the same concentration of DEA-NONOate before the treatment i.e. at baseline (Figure 3).

Discussion

A variety of natural products, including isolated compounds from plants, have been tested for treatment of male sexual dysfunction (21). Although guidelines do not give any specific recommendation for their use, natural extracts are potentially useful in the management and treatment of male sexual dysfunction (16). Ginseng has been tested for its therapeutic properties, which include improving sexual function (22), physical performance (23), treating cancer (24), diabetes (25) and hypertension (26). Data available suggest that ginseng has some testosterone-like effects and it could contribute to smooth muscle relaxation of the corpus cavernosum via NO pathway (27).

Moringa oleifera has been long used in traditional medicine. Many studies have reported its antioxidant, hypoglycaemic, anti-dyslipidaemia activities, tissue-protective (liver, kidneys, heart, testes, and lungs), analgesic, antihypertensive and immunomodulatory actions (28-30). *Rutin* is a flavonoid glycoside characterized by antioxidant, antidiabetic, anti-lipid peroxidation actions. In particular, data suggest that *rutin* has antioxidant activity and increases testosterone levels in diabetic condition in preclinical studies. Furthermore, it has been shown that *in vitro rutin* can inhibit PDE5 and arginase increasing the availability of NO and cGMP (31-33). This nutritional supplement formulation, containing a balanced content of *Moringa oleifera*, *rutin* and *ginseng*, has designed to act as an endothelial protector to be used as an adjuvant in the treatment of ED. The efficacy of the nutritional supplement has been tested in a clinical study by performing a combination therapy with a low dose of chronic Tadalafil regimen that has shown to improve the erectile function in vasculogenic patients. There were no significant differences between the groups in terms of baseline erectile function and presence of comorbidities.

The addition of a daily capsule of the nutritional supplement to Tadalafil 5 mg daily did not affect total testosterone, FSH, LH and PRL values. Although several animal and human studies suggested metabolic effects of *Moringa oleifera* and *rutin*, in our study,we did not found anti-dyslipidemic and hypoglycemic activity, probably because this was a chronic effects and 3 months could be a limited time to observe the effect. In addition, human studies showed that *Moringa oleifera* mainly determines a reduced post-prandial blood glucose levels and a long term reduction of HbA1C, rather than fasting blood glucose and we did not assess these parameters as it was not the purpose of our study (34). The clinic al evaluation indicates a statistically significant effect on sexual function. The IIEF-5 score increased in group A of 7.27 points vs 4.90 in group B with a significant difference of 2.37 points that represents a 20% increase over the placebo treatment. There was no significant difference in SEP2 and SEP3 values between Tadalafil plus nutritional supplement and Tadalafil plus placebo. Thus, the treatment with the formulation (*Panax ginseng*, *Moringa oleifera* and *rutin*) improves the IEEF-5 score. The second part of the study was performed to validate the data obtained by using the questionnaires. Indeed, as in this case, questionnaires are strongly biased by the placebo treatment. We have previously shown that platelet cGMP represents a suitable and objective biomarker of PDE5-inhibitors efficacy in ED clinical studies.

This evidence relies on the fact that i) PDE5-inhibitors act by enhancing the NO/cGMP signaling ii) PDE5 is present in human platelets (35, 36) iii) treatment with PDE5-inhibitors increases platelet cGMP levels (18, 37). In particular, we have demonstrated that, following chronic treatment with vardenafil 5 mg/daily of ED patients, the platelet cGMP levels were significantly increased and well correlated (significantly) with the VSS-Rigiscan measure-

ment (18). Thus, the measurement of platelet cGMP from blood samples of patients represents an unbiased marker of activity. The analysis of platelets harvested from patients treated with the nutritional supplement plus Tadalafil showed a significant increase in the cGMP levels when stimulated with DEA-NONOate 100 μM. This result suggests that the treatment with the nutritional supplement ameliorates and extends the activity of the chronic treatment with Tadalafil maintaining a significant more elevated levels of inhibition of PDE5. In this context, it is important to stress that there are several clinical pieces of evidence that PDE5-inhibitors effect can go beyond their half-life (18, 38). Indeed, clinical data reported that men still have facilitated erections when the levels of PDE5-inhibitors are below of the therapeutic plasmatic concentration (39-41).

Conclusions

In conclusion, the significant increase of the IIEF-5 can be ascribed to the nutritional supplement properties. Indeed, beyond the well-known antioxidant effects, *Moringa oleifera*, *ginseng* and *rutin*, it has been reported that can enhance the endothelial NO and cGMP production (41-43).

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