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Review

Bergamot polyphenolic fraction counteracts erectile dysfunction occurring in patients suffering from type 2 diabetes

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ABSTRACT

Erectile dysfunction (ED) is a complex disease occurring mainly in patients with metabolic disorders including Diabetes Mellitus and Metabolic syndrome. It correlates with an increased occurrence of cardiovascular disorders including coronary artery disease and heart failure. Alongside with the development of inhibitors of 5-PDE (sildenafil, tadalafil etc.), many medicinal plants used in traditional medicine have been claimed to produce potential benefit in approaching ED, though further clinical evidences are required in order to demonstrate their efficacy in counteracting such a disease state. Here, we summarize some of recent advances in treating ED in diabetic patients via bergamot polyphenolic fraction (BPF 47%), a natural antioxidant known to lower serum lipids and glucose. In addition, the effect of BPF alone or in combination with Tribulus Terrestris and Epimedium extract in supporting endothelial function and reactive vasodilatation has been explored after 120 day treatment via International Index of Erectile Function, Doppler penile flow detection and Endopat measurements, showing a significant beneficial effect in overall parametres studied to assess ED.

Thus, BPF alone or in combination with other medicinal plants represents a novel natural therapeutic option for counteracting ED in patients with cardiometabolic disorders, mostly due to its activity in supporting endothelial function and modulation of penile arteriolar blood flow.

Contents

1. Introduction

In the last decades, the use of medicinal plant extracts has significantly been supported aiming to get an alternative "natural" approach in the treatment of sexual dysfunction both in men and in woman. Despite promising preliminary results, mostly deriving from the traditional use of officinal plants supporting sexual performance in the era preceding the development of 5-phoshodiesterase inhibitors (5-PDEi such as sildenafil), plant-deriving extracts are still under investigation in order to prove relevant benefit while used in moderate to severe erectile dysfunction (ED).

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ED occurs in patients with cardiometabolic disorders and subsequent severe endothelial cell dysfunction (e.g. type 1 and type 2 diabetes) as well as in subjects undergoing surgical procedures in the genito-urinary tract.

ED is defined as "a condition of transient or persistent inability to achieve or maintain penile erection" [1]. The prevalence of sexual dysfunction has been assessed to be in the range of 2–10% in men younger than 40 years, 2–9% among men between 40 and 49 years, and it increased to 20–40% among men between 60–69 years, reaching the highest rate in men older than 70 years (50–100%). In the Massachusetts Male Aging Study [2], diabetic men showed a threefold probability of having ED than men without diabetes; moreover, the age-adjusted risk of ED was doubled in diabetic men compared with those without diabetes [3]. In addition, it has been estimated that the worldwide prevalence of ED will rise to 322 million cases by the year 2025 [4,5]. Several cross sectional and longitudinal studies showed an association between ED and most of the cardiovascular risk factors, such as diabetes [6–8], smoking [9], hypertension [10], hyperlipidemia [11], metabolic syndrome [12], lower urinary tract symptoms [13], and poor health state [14]. Moreover, ED is a marker of significantly increased risk of CVD [15], CHD, stroke, and all-cause mortality [16–18]. ED can be easily detected by having male patients complete standardized questionnaires investigating their sexual function. One of the most practical questionnaires that is administered is the International Index of Erectile Function (IIEF)-5 [19,20], which consists of items 5, 15, 4, 2, and 7 from the full-scale IIEF-15; a sum score of 21 or less indicates the presence of ED.

2. The association of ED with diabetes

Epidemiological studies suggest that both type 1 and type 2 diabetes are associated with an increased risk of ED, which is reported to occur in >50% of men with diabetes worldwide. (9.20) In the Massachusetts Male Aging Study [2], diabetic men showed a threefold probability of having ED when compared to men without diabetes; moreover, the age-adjusted risk of ED doubled in diabetic men when compared to those without diabetes [3] Most of the studies that described the prevalence of ED in diabetes did not distinguish between type 1 and type 2 diabetes. Two studies [21,22] reported a similar likelihood of having ED among both type 1 and type 2 diabetic men, whereas another report [23] showed a higher risk of developing ED in men with type 1 diabetes. The occurrence of ED is 10–15 years earlier in men with diabetes [12]; moreover, ED is more severe [24] and less responsive to oral drugs [25,26] in diabetes, leading to reduced quality of life [24,27].

Advanced age and longer duration of diabetes have been associated with an increased risk of ED in diabetic patients [23,28,29].

Whether hyperglycemia is a risk factor for the development of ED in diabetic men is still not clear. Some observational studies have shown an association between poor glycemic control, expressed by elevated levels of glycated hemoglobin (HbA1c) and ED, whereas other studies did not report any association [30,31].

The different methodological approaches used in the different studies may explain, at least in part, these divergent results. Moreover, diabetes is commonly associated with hypertension, hyperlipidemia, overweight and obesity, metabolic syndrome, smoking, sedentary lifestyles, and autonomic neuropathy, which are recognized as risk factors for ED [32,33]. Both microvascular [30,34,35] and macrovascular [36,37] diabetic complications also increase the risk of ED in diabetic men. The use of several medications frequently assumed by diabetic patients, such use of antihypertensive drugs (β-blockers, thiazide diuretics, and spironolactone), psychotropic drugs (antidepressants), and certain fibrates, have all been associated with an additive deleterious effect on diabetic ED [38,39]. A moderate consumption of alcohol (not more than 5% of the total daily caloric intake, or 7 alcoholic drinks per week) may exert a protective effect on ED in both the general population and in diabetic men [22].

3. Pathophysiology of ED in diabetic patients

The pathogenesis of ED in diabetes is multifactorial, as it depends on both psychological and organic factors (which play major roles in ED), as well as psychological and relationship issues, which often coexist. The proposed mechanisms of ED in diabetic patients are represented by vasculopathy, neuropathy, visceral adiposity, insulin resistance, and hypogonadism.

Diabetic vasculopathy concerns macroangiopathy, microangiopathy, and endothelial dysfunction. Macrovascular disease in diabetes corresponds to the atherosclerotic damage in the blood vessels, which limits blood flow to the penis. As mentioned, several cardiovascular risk factors associated with diabetes contribute to the genesis of penile arterial insufficiency [9,10]: all of them converge on endothelial dysfunction, which represents the common denominator leading to vascular ED.

The chronic insult of hyperglycemia on the endothelium results in endothelial dysfunction, which has been suggested as the link between ED and CVD. A diagnosis of ED may be seen as a sentinel event that should prompt the investigation of coronary artery disease (CAD) in asymptomatic diabetic men [40]. Endothelial dysfunction in diabetes is manifested as the decreased bioavailability of nitric oxide (NO), resulting in insufficient relaxation of the vascular smooth muscle of the corpora cavernosa. The potential mechanisms involved in endothelial dysfunction include the accumulation of advanced glycation end products; increased levels of oxygen free radicals that reduce the bioavailability of NO; impaired endothelial and neuronal NO synthesis, expression, and activity; and an imbalance between the vasoconstrictive and vasorelaxant intracellular pathways favoring increased vasoconstriction [41]. Esposito et al. [42] observed an increase in circulating endothelial microparticles – an emergent marker of endothelial dysfunction – in diabetic men with ED, as compared with nondiabetic men.

Microvascular disease determines ischemic damage in the distal circulation and autonomic and peripheral neuropathy. Both somatic and autonomic neuropathies may contribute to diabetes-induced ED due to the impairment of sensory impulses from the penis to the reflexogenic erectile center [43], and reduced or absent parasympathetic activity necessary for relaxation of the smooth muscle of the corpus cavernosum [44].

Insulin resistance and visceral adiposity, which are both distinctive clinical traits of type 2 diabetes, are associated with a proinflammatory state that results in the decreased availability and activity of NO, leading to ED in overweight and obese diabetic men.

Subnormal testosterone concentrations have been found in 25% of men with type 2 diabetes in association with inappropriately low luteinizing hormone (LH) and follicle- stimulating hormone (FSH) concentrations [45,46]. Testosterone regulates nearly every component of erectile function, from pelvic ganglions to smooth muscle, and to the endothelial cells of the corpora cavernosa. It also modulates the timing of the erectile process, which occurs as a function of sexual desire, coordinating penile erection with sex. It is still unclear what level of testosterone is needed for good erectile function; however, evidence derived from clinical and molecular studies supports the use of testosterone replacement in hypogonadal patients with ED, although the benefit–risk ratio is uncertain in advanced age [47,48]. The mechanisms involved in

testosterone deficiency in diabetes include low levels of the sex hormone-binding globulin due to insulin resistance, increased aromatase activity in visceral adipose tissue lead- ing to an augmented conversion of testosterone in estradiol, leptin resistance causing reduced secretion of LH and testosterone, and increased levels of inflammatory mediators, which may suppress the secretion of gonadotropin-releasing hormone and LH [49]. Bellastella et al. [50] suggested a possible autoimmune pathogenesis of hypogonadotropic hypogonadism in type 2 diabetic patients, as indicated by the presence of antipituitary antibodies at high titers, as compared with age-matched controls.

4. The effect of bergamot polyphenolic fraction in ED in diabetic patients

Recently, evidence has been collected that bergamot polyphenolic fraction (BPF), due to its antioxidant properties, produces an enhancement of NO release from endothelial cells in patients suffering from mixed hyperlipemia and diabetes. In particular, we have shown that flow-mediated hyperemia, a reliable methodology to studying endothelial function in patients, which is impaired in diabetes and/or hyperlipemia, is improved by administration of BPF (500–1000 mg/daily) [51]. In particular, endothelial function was measured from brachial artery flow-mediated vasodilatation with B-mode ultrasound imaging of the brachial artery and by assessing the increase in artery diameter during reactive hyperemia.

Before starting the BPF treatment, flow-mediated vasodilation was found reduced in patients suffering from isolated hyperlipemia as well as hyperglicemia.

After 30 days of BPF treatment with (500 and 1000 mg/daily for 30 consecutive days), flow-mediated vasodilation increased significantly, whereas no changes have been observed in patients receiving placebo. This suggests that BPF is able to improve the impaired endothelium-mediated vasodilation in hyperlipidemic patients with or without hyperglycemia.

On the basis of these preliminary results, we recently studied the effect of 650 mg twice a day of 47% BPF for 120 consecutive days in 40 diabetic patients with mild to severe ED. Here we report part of the results of a randomized placebo-controlled clinical study. All participants provided written informed consent prior to participation. The study was approved by the local Medical Ethics Committee at San Raffaele IRCCS (Rome, Italy) and all the patients enrolled in the study provided written informed consent prior to participation.

For the preparation of BPF, bergamot juice was obtained from peeled-off fruits by squeezing. The juice was oil fraction-depleted by stripping, clarified by ultra-filtration and loaded on to a suitable polystyrene resin column able to absorb polyphenol compounds of molecular weight between 300 and 600 Da (Mitsubishi). Polyphenol fractions were eluted by a 1 mM KOH solution. The basic eluate was incubated at a rocking platform to reduce the furocumarin content.

The shaking time was adjusted proportionally to the amount of furocumarin contaminants. Next, the phytocomplex derived from the process performed to remove furocumarins was neutralized by filtration on cationic resin at acidic pH. Finally it was vacuum dried and minced to the desired particle size to obtain BPF powder.

BPF powder was analysed for flavonoid, furocumarin and other polyphenol content which was standardized at 38%. In addition, all toxicological analyses were performed, including heavy metal, pesticide, phthalate and synephrine content which revealed the absence of known toxic compounds at significant levels.

Standard microbiological test showed the final BPF was free of mycotoxins and contaminating bacteria. Finally, 650 mg aliquots of the BPF powder were encapsulated together with 100 mg oleuropein 20% and 50 mg of ascorbic acid into suitable gelatin capsules by a semi-automatic gelatin encapsulation device employing an authorized pharmaceutical manufacturer (Plants, Messina, Italy). All procedures have been performed according to Good Manufacture Practice (GMP) headlines of European Legislation.

Table 1
Baseline characteristics of diabetic patients entering the study.

	PLACEBO	Diabetic patients (DP)
Number	20	20
Age	52 ± 4	54 ± 6
BMI	29 ± 3	30 ± 2
Total cholesterol	172 ± 8	191 ± 9
Serum glucose	90 ± 4	118 ± 8
HbA1	5.8 ± 0.3	7.2 ± 0.4
BP sistolic	120 ± 8	128 ± 8
BP diastolic	80 ± 4	82 ± 5
Triglicerydes	154 ± 12	168 ± 10
smokers	0	0

Tablets containing 1000 mg of maltodestrin supplemented with 50 mg ascorbic acid were used as placebo.

In addition, the study was carried out according to the European Guidelines for Good Clinical Practice procedures. Demographic characteristics of patients undergoing placebo or BPF treatment are summarized in Table 1. In particular, patients were classified according to the International Index of Erectile Function (IIEF) and were found suffering from mild to severe ED (IEEF 12 ± 4) (Table 2).

The occurrence of ED in such diabetic patients has also been assessed by measuring peak systolic velocity in penile artery by means of Doppler analysis [52].

It is known that Pulsed Doppler Flow measurements are currently used to examine the cavernosal arteries and the response of their spectral Doppler waveforms following intracavernosal injection of a pharmacostimulant agent such as alprostadil. The fundamental principle is repeated sampling of these waveforms in a stepwise manner until maximal peak systolic and minimal diastolic velocities have been reached [52].

Repeated Doppler measurements should occur at 5-min intervals until the maximal peak systolic velocity (PSV) and end-diastolic velocity (EDV) are judged to have been reached. The PSV is normal if it is >35 cm s^{-1} and EDV is usually normal if negative or close to 0 cm s^{-1}. In diabetic patients enrolled for the study, the mean PSV was found to be 22 ± 6 cm s^{-1}, thus suggesting that an impaired regulation of penile circulation was accompanying ED (Fig. 1).

The impaired endothelial function occurring in patients with diabetes was also found by means of EndoPAT measurements, a reliable methodology for assessing endothelial-mediated regulation of blood flow in humans [53].

In particular, the procedure involves the patient being placed in the supine position with a blood pressure cuff secured on the upper

Table 2
The effect of BPF on the International Index of Erectile Function (IIEF) of diabetic patients (DP).

CTRL	DP	DP + BPF
27 ± 3	12 ± 4*	18 ± 3§

International Index for Erectile Function (IEEF)

- Normal: IIEF from 26 to 30.
- Low grade ED: IIEF from 17 to 25.
- Mild ED: IIEF from 11 to 16.
- Severe ED: IIEF from 6 to 10.

* $p < 0.05$CTRL vs DP.
§ $p < 0.05$ DP vs DP + BPF.

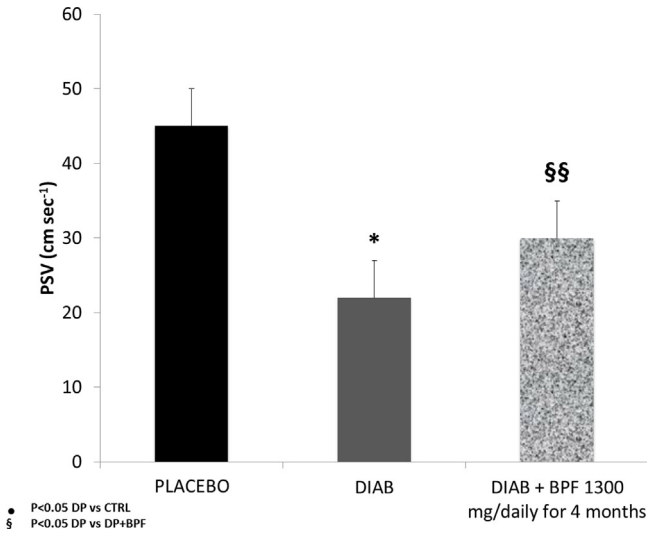

Fig. 1. The effect of bergamot polyphenolic fraction (BPF—1300 mg/daily for 4 consecutive months) on Doppler Peak Systolic Velocity (PSV cm s^{-1}) in dorsal penile artery of diabetic patients.

arm. The room was quiet, temperature controlled, and dimly lit for comfort. PAT probes were stationed on the index finger of each hand. After a 3–5 min lead-in equilibration time to evaluate baseline parameters, the blood pressure cuff was inflated to a level sufficient enough to inhibit arterial pulsations as recorded by the PAT probe (average value ~200–220 mmHg). The cuff was inflated to occlusion pressures for 5 min to allow adequate end-tissue hypoxia. Following this, the cuff was released and values recorded for the following 3–5 min (the hyperemic period). The values during this time constituted the Reactive Hyperemia Index (RHI). RHI values were normalized to the control arm (the arm without the blood pressure cuff) to compensate for possible systemic changes. As a measurement of endothelial dysfunction in medium/small arteries, the RHI values werv cx e interpreted as those <2 exhibiting endothelial dysfunction and those >2 being normal. On the other hand, arterial stiffness was also obtained via PAT through measurement of the augmentation index (AI). AI was calculated automatically from analysis of the PAT waveform and functions as a measure of medium/large arterial wall elasticity [54]. Normal arterial stiffness is defined by an AI of −30 to −10%, increased arterial stiffness from −10% to +10% and abnormal arterial stiffness >10%. In diabetic patients entering the study, the RHI value was 1.52 ± 1.2, the derived Framingham RHI was 0.24 ± 0.3 and the AI was −6.2 ± 2.2, thus demonstrating that endothelial function is deteriorated in diabetic patients with ED (Table 3).

The treatment with BPF 47% (650 twice a day for 120 consecutive days) significantly improved ED as assessed by means of IIEF score. In fact, after 120 day treatment with BPF IIEF was improved from baseline 12 ± 4 to 18 ± 3 (Table 2). This effect was accompanied by and improvement of Doppler PSV as measured after intracavernosal injection of alprostadil. Indeed, PSV was found 29 ± 5 cm s^{-1} after BPF treatment, thus confirming that the highly concentrated polyphenolic extract of bergamot (47%) clearly enhances arterial blood flow in penile arteries (Fig. 1). Moreover, the enhancement of endothelial function was also found to contribute in restoring a satisfactory sexual function in diabetic patients. Indeed, both HRI, fHRI and AI, as detected by means of EndoPAT methodology, were found improved after treatment with BPF 47% in diabetics (Table 3).

No relevant side effects were described throughout and at the end of the study. None of the patients enrolled in the study interrupted the treatment.

Thus, bergamot-derived highly concentrated polyphenolic fraction (BPF 47%) partially restores erectile function in diabetic patients, mostly via enhanced ability of endothelial cells to release vasoprotective factors. This fits very well with many evidences collected from our and other groups suggesting that BPF, due to its antioxidant effect, leads to vasoprotective action, and that this effect is accompanied by increased expression of endothelial biomarkers including Phospho-PKB as well as constitutive nitric oxide-synthase [54]. On the other hand, BPF attenuates oxyLDL-induced expression of LOX-1, the scavenger receptor involved in oxy-LDL atherogenic activity, thus indicating that overall effect of BPF in hyperlipemic and diabetic patients is consistent with a multifactorial improvement of endothelial function [54].

The positive effect of BPF 47% in patients with ED is highlighted when combined with natural products other than citrus-derived polyphenols claimed to improve sexual performances in diabetics as well as in non-diabetic subjects suffering from ED.

In particular, we recently found that 10 diabetic patients receiving a combination (Bergamale, Nathealth Solutions, USA) of BPF 47% (500 mg/twice a day) with Bulgarian Tribulus terrestris extract (200 mg/twice a day) and 150 mg of 20% Epimedium extract, significantly increased the number of spontaneous nocturnal erections (SNE; from basal 4 ± 2 to 18 ± 3 montly erections). This effect occurred when Bergamale was added for 30 days to conventional treatment with BPF 47% alone and was accompanied by a mean 30 ± 4% improvement of both IIEF score and EndoPAT parametres, suggesting a synergistic effect of BPF 47% with tribulus and epimedium extracts. The reason of these results still remains to be clarified. However, evidence exists that Bulgarian Tribulus extract enhances testosterone levels in patients with ED. In addition, some components of epimedium extract (icarine and icariside, in particular) have been proven to inhibit 5-PDE enzyme in a way similar to the one of sildenafil (VIAGRA_like effect). Thus, it is likely that both these effects combined with the enhanced endotelial performance produced by BPF 47% leads to synergistic action able to better counteract ED occurring in patients undergoing metabolic syndrome and diabetes.

5. Conclusion

ED is a complex disease which occurs mainly in patients suffering from metabolic syndrome and diabetes. The impairement of sexual performance in such patients seems to occur very early in the course of the disease being mainly due to an impaired endothelial cell functionality and to an altered metabolism of

Table 3
The effect of BPF (1300 mg/daily for 4 consecutive months) on EndoPAT scores of diabetic patients (DP).

	CTRL	DP	DP + BPF
Reactive hyperemia index (RHI)	2.2 ± 0.18	1.52 ± 1.2*	1.90 ± 1.6§
Framingham reactive hyperemia index (fRHI)	0.35 ± 0.2	0.24 ± 0.3*	0.30 ± 0.3§
Augmentation index (AI)	−28.7 ± 4.2	−6.2 ± 2.2*	−16 ± 3.2§

* $p < 0.05$ DP vs CTRL.
§ $p < 0.05$ DP vs DP + BPF.

testosterone in the liver. Both activities are significantly improved by regular administration of higly concentrated bergamot-derived polyphenolic fraction (BPF 47%), a natural antioxidant proven to counteract endothelial dysfunction. Furthermore, the activity of BPF 47% seems to synergize with plant extracts such as Tribulus Terrestris and Epimedium, able to restore testosterone levels and to inhibit 5-PDE, respectively.

Thus, bergamot extract alone or in combination with plant extracts proven to be effective in treating sexual dysfunction, counteracts ED thereby contributing in getting a better vaso-protection in patients with cardiometabolic disorders.

Conflict of interest

The authors declare no conflict of interest.

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