Neuroendocrinology Letters Volume 38 No. 1 2017
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

Comparative effects of a fixed *Polypodium leucotomos*/Pomegranate combination versus *Polypodium leucotomos* alone on skin biophysical parameters

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Submitted: 2017-01-27 Accepted: 2017-02-12 Published online: 2017-00-00

Key words: ***Polypodium leucotomos*; pomegranate; skin biophysical parameters; supplementation; photoprotection**

Neuroendocrinol Lett 2017; **38**(1):101–105 **PMID:** ----- NEL380117AXX

Abstract

OBJECTIVES: *Polypodium leucotomos* extract is a commonly used systemic photoprotective agent. In an exploratory fashion, the current study aimed to compare the effects of oral supplementation with a fixed *Polypodium leucotomos*/pomegranate combination (PPmix®) versus *Polypodium leucotomos* alone (Fernblock®) on skin biophysical parameters of Caucasian adults.

METHODS: Forty healthy adult volunteers (20 males and 20 females; mean age: 37.2±5.5 years) were randomized in a 1:1 fashion to a fixed *Polypodium leucotomos*/pomegranate combination (480 mg/day; n=20) or *Polypodium leucotomos* alone (480 mg/day; n=20) for 3 months. Six skin biophysical parameters (skin sebum content, hydration, transepidermal water loss [TEWL], erythema index, melanin index, and elasticity) were measured at baseline and after 3 months by personnel blinded to participant allocation.

RESULTS: At the end of the study, hydration and elasticity were significantly improved and TEWL was reduced in both groups, without significant intergroup differences. The erythema index was decreased by both treatments, although the fixed *Polypodium leucotomos*/pomegranate combination was significantly more effective. Finally, melanin index and skin sebum content were reduced by the fixed Polypodium leucotomos/pomegranate combination, whereas *Polypodium leucotomos* alone did not affect them.

CONCLUSIONS: Our results suggest that a fixed *Polypodium leucotomos*/pomegranate combination provides a greater improvement of skin biophysical parameters compared to *Polypodium leucotomos* alone in adult Caucasians. Our findings may have implications for optimizing systemic skin photoprotection and beautification strategies.

ORIGINAL ARTICLE

INTRODUCTION

Exposure of the skin to ultraviolet radiation (UVR) may directly produce varying levels of free radical formation and also deplete or inhibit to various degrees levels of endogenous antioxidants (Katta & Brown 2015; Emanuele *et al.* 2014). Consequently, supplementation of skin with antioxidants may be useful to compensate for their UVR-induced depletion (Fernández-García 2014), ultimately preventing oxidative damage to DNA and proteins in different skin cell types (Emanuele *et al.* 2014). The extract of *Polypodium leucotomos* – a species of fern in the Polypodiaceae family found in South America – is one of the most common oral supplements used for systemic photoprotection (Gonzalez *et al.* 2007; Choudhry *et al.* 2014; El-Haj & Goldstein 2015; Winkelmann *et al.* 2015; Bhatia 2015; Palomino 2015; Parrado *et al.* 2016). Research in healthy volunteers has shown that oral administration of *P. leucotomos* for two days is sufficient to protect the skin against subsequent exposure to artificial UVR (Middelkamp-Hup *et al.* 2004). Molecular studies demonstrated that *P. leucotomos* can decrease erythema following acute UVR exposure effect mainly by scavenging reactive oxygen species (ROS), ultimately mitigating UVR-induced formation of oxidized DNA bases (Zattra *et al.* 2009). Because long-term administration of *P. leucotomos* does not pose safety concerns (Nestor *et al.* 2015; Murbach *et al.* 2015), the extract has been also tested in a variety of skin conditions, including atopic dermatitis (Ramírez-Bosca *et al.* 2013) and as an adjunct in the treatment of pigmentation disorders – including vitiligo and melasma (Nestor *et al.* 2014). Although results have been generally promising, the mechanisms underlying the potential utility of *P. leucotomos* in these conditions have not yet been completely elucidated (Berman *et al.* 2016).

Apart from *P. leucotomos,* the characteristic biochemical composition of pomegranate fruit (*Punica granatum*) – being rich in antioxidants and anti-inflammatory polyphenols (e.g., punicalagin and other ellagitannins) (Syed *et al.* 2013; Johanningsmeier & Harris 2011) – has drawn attention in skin photoprotection and inhibition of photocarcinogenesis (Baccarin *et al.* 2015). Recent studies found that topical application of *P. granatum* extract can downregulate proinflammatory molecules – including COX-2 – both in an *ex vivo* model of porcine skin (Houston *et al.* 2016) and when administered orally to mice (Khan *et al.* 2012). *In vitro* experiments also demonstrated that *P. granatum* extract inhibits tyrosinase activity and melanin production (Rana *et al.* 2013). In addition, *P. granatum* concentrated solution has been shown to enhance hyaluronan synthesis, as well as suppress elastase, collagenase, and metalloproteinase (MMP)-1 activity, potentially exerting significant cosmetic effects (Kang *et al.* 2015).

In light of these findings, we hypothesized that a combination of *P. leucotomos* and *P. granatum* might exert additive or synergistic effects compared to the widely used *P. leucotomos* alone for systemic skin care applications. The current study was therefore designed to compare in an exploratory fashion the effects of oral supplementation with a fixed *Polypodium leucotomos*/pomegranate combination (PPmix®) *versus Polypodium leucotomos* alone (Fernblock®) on skin biophysical parameters of healthy Caucasian adults.

METHODS

Study participants

The study population comprised 40 volunteers aged >18 years (20 males and 20 females; mean age: 37.2±5.5 years) in apparently good physical health. All participants were of Caucasian descent (Fitzpatrick skin phototype II–III) and were free of any known dermatological conditions. Subjects with a history of significant neurologic, psychiatric, hematological, endocrine, cardiovascular, respiratory, renal, hepatic, or gastrointestinal disease, or coagulation deficits were excluded. Women who were pregnant or breastfeeding were also ineligible. The study was approved by the local ethics committee and complied with the tenets of the Declaration of Helsinki. Before the study, each participant was informed in detail about the purpose of the research, and signed informed consents were obtained.

Supplements

P. leucotomos extract (Fernblock®) was from IFC Group (Madrid, Spain). The fixed *P. leucotomos*/pomegranate combination (PPmix®) was purchased from Bioenx (Florence, Italy). The preparation – in form of a red-brownish hygroscopic powder – was obtained through a patented proprietary technology by spraying *P. leucotomos* powder with pomegranate juice containing polyphenols (punicalagin and other hydrolyzable tannins, gallic acid, anthocyanins, flavones). The relative percentages of *P. leucotomos* and pomegranate in the fixed *P. leucotomos*/pomegranate combination are covered by industrial intellectual protection.

Allocation

All participants were asked to withdraw any topical skin product 14 days before the beginning of the study. In addition, they were not allowed to use any topical skin intervention throughout the entire study period. With the use of a computer-generated random-allocation sequence, patients were assigned in a 1:1 fashion to receive tablets containing a proprietary fixed *P. leucotomos*/pomegranate combination (480 mg/day, n=20) or *P. leucotomos* alone (480 mg/day, n=20) for 3 months. Both the investigators and the study participants were blinded to supplement allocation.

Outcome measures

There were six skin biophysical parameters targeted as outcome measures in the study: skin sebum content,

Tab. 1. Changes from baseline to 3 months in skin biophysical parameters in two study groups.

	Fixed *Polypodium leucotomos*/pomegranate combination (n=20)		*Polypodium leucotomos* alone (n=20)	
	Baseline	**3 months**	**Baseline**	**3 months**
Skin sebum content	53.6±15.2	44.1±18.4*,†	55.0±13.6	57.1±14.5
Hydration	50.1±11.8	59.3±14.6*	51.7±12.9	60.1±18.3*
TEWL	14.4±5.3	10.3±4.2*	13.6±5.9	9.9±4.8*
Erythema index	359±101	287±93*,†	351±98	311±103*
Melanin index	175±48	154±60*,†	180±53	174±58
Elasticity	0.30±0.11	0.37±0.16*	0.33±0.13	0.41±0.19*

TEWL, transepidermal water loss. *p<0.001 versus baseline; †p<0.001 versus *P. leucotomos* alone.

hydration, transepidermal water loss (TEWL), erythema index, melanin index, and elasticity. All measurements were performed at baseline and repeated after 3 months of supplementation.

Assessment of skin biophysical parameters

All skin biophysical parameters were measured in the same anatomical location (right cheek) by personnel blinded to the allocated supplement. All assessments were performed at room temperature (20–25 °C) with a relative humidity of 40–50% according to previously described protocols (Firooz *et al.* 2012). Skin sebum content was measured with a Sebumeter SM 815 (Courage & Khazaka electronic GmbH, Cologne, Germany) and expressed as μg/cm^2. Hydration was assessed using a Corneometer CM 825 and the results were expressed using system-specific arbitrary units. TEWL was quantified with a TEWAmeter TM 300 and was expressed as g/m^2/h. The erythema index was calculated with Mexameter MX 18 from the strength of the absorbed and the reflected light at 568 and 660 nm, respectively. The melanin index was determined in a similar manner at 660 and 880 nm, respectively. Finally, skin elasticity was measured with a cutometer MPA 580 and expressed in arbitrary units.

Statistical analysis

Data are given as means ± standard deviations or counts, as appropriate. Categorical data were analyzed with the χ^2 test. Unpaired Student's t-tests were performed to compare the continuous variables at baseline. One-sample paired Student's t-tests were used for within-group comparisons between baseline and post-treatment skin biophysical parameters. Linear mixed models were utilized to detect potential interactions which might influence the relation between treatment and change in the study variables (including age and sex). All calculations were performed using the SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). Two-tailed p values <0.05 were considered statistically significant.

RESULTS

The mean age of subjects in the fixed *P. leucotomos*/pomegranate combination group was 37.5±5.9 years (age range: 29–50 years) and was not significantly different from that observed in the *P. leucotomos* alone group (37.1±5.3 years; age range: 29–48 years; p=0.80). The fixed *P. leucotomos*/pomegranate combination group comprised 9 males and 11 females, whereas the *P. leucotomos* alone group consisted of 11 males and 9 females (χ^2=0.10, p=0.75). No participant withdrew from the study and no adverse events were reported.

There were no statistically significant differences in baseline skin biophysical parameters between the two study groups (all p=ns). Table 1 shows the changes in skin biophysical parameters in the two study arms after 3 months of supplementation. At the end of the study, hydration and elasticity were significantly improved and TEWL was reduced in both groups, without significant differences between the two arms. The erythema index was decreased by both treatments, although the fixed *P. leucotomos*/pomegranate combination was significantly more effective (p<0.001 *versus P. leucotomos* alone). Finally, melanin index and skin sebum content were reduced by the fixed *P. leucotomos*/pomegranate combination (both p<0.001 *versus* baseline), whereas *P. leucotomos* alone did not affect them. Linear mixed models showed no interactions between age, sex, and any of the observed changes in skin biochemical parameters.

DISCUSSION

The central question addressed in this exploratory pilot study was whether a fixed *P. leucotomos*/pomegranate combination and *P. leucotomos* alone could display different effects on skin biochemical parameters when orally administered to adult healthy Caucasian individuals. Our main results were as follows: 1) the fixed *P. leucotomos*/pomegranate combination and *P. leucoto-*

mos were equally effective in improving hydration and elasticity, as well as in reducing TEWL; 2) the erythema index was significantly decreased in both arms, albeit the fixed *P. leucotomos*/pomegranate combination was significantly more effective; and 3) the melanin index and skin sebum content were significantly reduced by the fixed *P. leucotomos*/pomegranate combination, whereas *P. leucotomos* alone did not change these parameters.

Reduced skin hydration and elasticity are well-known markers of skin aging and are strongly related to decreased dermal collagen content (Choi *et al.* 2013). Besides loss of cutaneous hydration and elasticity, a higher TEWL – which reflects an increased cutaneous evaporation rate – is a common age-related skin problem that could ultimately lead to xerosis (Luebberding *et al.* 2013). Improvements in skin elasticity and moisture content – as observed in our study – suggest that long-term supplementation with either a *P. leucotomos*/pomegranate combination or *P. leucotomos* alone could contribute to maintain a youthful skin appearance. Although further investigations are needed, it could be speculated that such positive effects could be related to an increase of dermal matrix macromolecule biosynthesis and/or inhibition of MMPs. Although such effects were demonstrated for both *P. leucotomos* (Berman *et al.* 2016) and pomegranate extract (Kang *et al.* 2015) separately, no synergistic or additive effects of their combination were evident in terms of skin hydration, elasticity, and TEWL in the current study. In contrast, our results indicated that *P. leucotomos*/pomegranate combination or *P. leucotomos* alone displayed significant differences in terms of erythema and melanin indexes. As far as the erythema index is concerned, the study groups showed both a significant reduction, with improvements being significantly superior in subjects who received the fixed *P. leucotomos*/pomegranate combination. A more marked decrease in the skin erythema index in these participants may also be explained by additive effects exerted by pomegranate (as compared with *P. leucotomos* alone) on skin hyperemia and/or inflammation. Interestingly, the melanin index – which reflects the extent of skin pigmentation – was unaffected by supplementation with *P. leucotomos* alone but decreased significantly in subjects who received *P. leucotomos*/pomegranate combination. These observations suggest that active compounds present in the pomegranate component of the combination were responsible for the reduction of skin pigmentation, with punicalagin being the most plausible candidate (Rana *et al.* 2013). These results may pave the way for the use of the *P. leucotomos*/pomegranate combination in systemic skin lightening products. We are nonetheless aware that baseline skin pigmentation levels may be a confounding factor when pigmentary skin changes are assessed (Firooz *et al.* 2012). Because the present study included Caucasian subjects with limited range of skin phototype (II–III), further investigations will be needed to establish whether the same effect could be detected in different skin types. Finally, we observed that skin sebum content was significantly decreased by the *P. leucotomos*/pomegranate combination but not by *P. leucotomos* alone. An increased sebum production is associated with hyperplasia of the sebaceous glands and represents one of the major concurrent events associated with the development of acne (Zouboulis 2004). Interestingly, the biological function of sebocytes is stimulated by several factors – including activation of peroxisome proliferator-activated receptors (Trivedi *et al.* 2006) – which are known to be downregulated by pomegranate extract (Hontecillas *et al.* 2009). Additional research is therefore needed to disentangle the exact molecular mechanisms by which sebum production is decreased by the *P. leucotomos*/pomegranate combination.

Our findings need to be interpreted in the context of some limitations. First, it is known that skin biophysical parameters vary with age, sex, body site, and ethnicity (Firooz *et al.* 2012). In our study, age and sex were well-matched in the two study arms and their confounding impact on our results is likely to be minor, if any. To minimize the effect of body site, all measurements of skin biophysical parameters were performed in the right cheek. Second, analysis of human skin explants was not performed in the current report, and the histological changes that might be responsible for the observed biophysical effects of supplementation deserve further scrutiny. Finally, our study was limited to Caucasian individuals and might not be generalizable to other ethnic groups.

In summary, our results suggest that a fixed *P. leucotomos*/pomegranate combination provides a greater improvement of skin biophysical parameters compared to *P. leucotomos* alone in adult Caucasians. Our findings may have implications for optimizing systemic skin photoprotection and beautification strategies.

Conflicts of interest

Enzo Emanuele is a shareholder of Bioenx srl. All other authors certify that there is no conflict of interest with any financial organization regarding the material discussed in this manuscript.

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