GYNECOLOGICAL ENDOCRINOLOGY
https://doi.org/10.1080/09513590.2020.1722994

Taylor & Francis
Taylor & Francis Group

REVIEW

Purified and specific cytoplasmic pollen extract: a non-hormonal alternative for the treatment of menopausal symptoms

Andrea Genazzani[a], Nick Panay[b], Tommaso Simoncini[c], Herman Depypere[d], Alfred Mueck[e,f], Christian Egarter[g], Nicoletta Biglia[h], Tomas Fait[i], Martin Birkhaeuser[j], Sven O. Skouby[k], Mark Brincat[l], Steven Goldstein[m], Xiangyan Ruan[f,e], Cuauhtémoc Celis-Gonzales[n] and Santiago Palacios[o]

[a]Department of Gynecology and Obstetrics, University of Pisa, Pisa, Italy; [b]Queen Charlotte's & Chelsea and Westminster Hospitals and Imperial College, London, The International Menopause Society, London, UK; [c]Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy; [d]Breast Clinic and Menopause Clinic, University Hospital, Gent, Belgium; [e]Department of Women's Health, University Hospitals of Tuebingen, Tuebingen, Germany; [f]Department of Gynecological Endocrinology, Beijing Obstetrics and Gynecology Hospital, Capital Medical University, Beijing, China; [g]Department of Obstetrics and Gynecology, Division of Gynecologic Endocrinology and Reproductive Medicine, University of Vienna, Vienna, Austria; [h]Academic Division of Gynecology and Obstetrics, Mauriziano Hospital, University of Turin, Turin, Italy; [i]Department of Obstetrics and Gynecology, Faculty Hospital Motol, 2nd Faculty of Medicine, Charles University, Prague, Czech Republic; [j]Department Obstetrics and Gynecology, Division of Gynecological Endocrinology and Reproductive Medicine, University of Bern, Bern, Switzerland; [k]Department of Gynecology and Obstetrics, Herlev University Hospital, Copenhagen, Denmark; [l]Department of Obstetrics & Gynecology, Mater Dei Hospital, Msida, Malta; [m]New York University School of Medicine, New York, NY, USA; [n]Federación Mexicana de Colegios de Ginecología y Obstetricia (FEMECOG), Mexico, Mexico; [o]Palacios Institute of Women's Health, Madrid, Spain

ABSTRACT

Research into non-hormonal, alternative therapies is necessary for women for whom menopausal hormone therapy is contraindicated or for women who do not wish to take hormones. This review focuses on one such non-hormonal option, namely, purified and specific cytoplasmic pollen extract, or PureCyTonin®. This extract has been evaluated in several preclinical and clinical studies, where it demonstrated its value as a safe and non-estrogenic alternative for menopause. This review presents the beneficial effects of PureCyTonin® in the treatment of menopausal symptoms (e.g. hot flushes) in healthy women, as well as in premenstrual syndrome. We discuss the mechanism of action of PureCyTonin®, an SSRI-'like' therapy. The lack of estrogenic effect demonstrated in preclinical studies suggests that PureCyTonin® may also be a suitable option for the management of menopausal symptoms in women with breast cancer.

ARTICLE HISTORY
Received 4 December 2019
Revised 14 January 2020
Accepted 25 January 2020
Published online 4 February 2020

KEYWORDS
Purified and specific cytoplasmic pollen extract; PureCyTonin®; menopause; non-hormonal alternative; hot flushes; premenstrual syndrome; Serelys®

Introduction

The life expectancy of women in developed countries has increased by more than 30 years during the last century. Consequently, menopause has become a natural event in a woman's life. Menopause is characterized by loss of reproductive function and decline in estrogen levels [1,2] and may be associated with multiple symptoms such as hot flushes, night sweats, sleep disturbances, decreased libido, sexual dysfunction, poor memory, anxiety/depression, and urinary incontinence, all of which may constitute indications for therapeutic intervention. Hot flushes are a thermoregulatory disorder due to a lack of estrogens. They are the most characteristic and distressing symptoms of menopause and have been reported to affect over 75% of middle-aged women [3]. Menopausal hormone therapy (MHT) is an established and effective option for hot flushes in menopausal women. However, it is contraindicated in conditions such as estrogen-dependent diseases and acute thromboembolic events. Adherence to hormone therapy is frequently not satisfactory. Since the primary and negative outcomes of the large-scale US clinical trial MHT of the Women's Health Initiative (WHI) Study in 2002, the number of MHT prescriptions has dropped drastically.

While regulatory bodies in Europe and the USA recommend that MHT should be used for as short a time as possible, some clinical guidelines recommended less restrictive prescribing. However, for women for whom MHT is contraindicated or who do not wish to take hormones, research into alternative therapies is clearly necessary. These include non-hormonal options, which may be non-pharmacological or pharmacological. Non-pharmacological interventions include lifestyle changes, behavioral therapies, holistic techniques, food supplements, and herbal therapies [4]. Pharmacological interventions include anti-depressants, anti-hypertensive drugs, and anti-epileptics [5,6].

This review focuses on PureCyTonin®, which is the main ingredient of the food supplement Serelys®. Serelys® is natural in origin and constitutes a non-hormonal alternative for the treatment of hot flushes and other menopausal symptoms. Its value and safety in the treatment of menopausal hot flushes and in premenstrual syndrome (PMS) has been demonstrated in several preclinical *in vitro* and *in vivo* studies and in clinical trials.

CONTACT Andrea Genazzani argenazzani@gmail.com Department of Gynecology and Obstetrics, University of Pisa, Pisa, Italy

Background

Pollen (from the Greek παλη [pale]: flour or dust) constitutes the male gametophyte of the flower. It is composed of minuscule ovoid grains measuring 20–55 µm in diameter that are initially contained at the end of stamens and are surrounded by a shell called an exine. The function of this pollen shell is to protect the genetic material. Well-known allergens are located on the external shell, whereas the center of the pollen contains the cytoplasm [7]. Germination occurs when the pollen is transported by wind or bees to a compatible female pistil. The male gametophyte produces a pollen tube that transfers the sperm to the egg, which contains the female gametes. Pollen has long been used for therapeutic purposes by various civilizations (e.g. Ancient Egypt and China, as far back as 200 BC). The chemical composition of pollen depends largely on the plant source and geographic origin, together with factors such as climatic conditions, soil type, and the activity and race of the bees. However, since pollen may be of unspecific origin (from unknown plants) and may be mixed with other bee products, standardization is difficult [8]. In addition, when the grain is unopened, its components can be partially assimilated because the pollen shell cannot be damaged by stomach acid [9,10]. The shell proteins are meant to be highly allergenic [9,10]. Gôsta Carlsson, a Swedish gynecologist, is considered the 'father' of the pollen extraction method. In the 1940s, he took an empirical approach to this field, with the hypothesis that the administration of pollen extracts could return strength and vigor to elderly patients.

Production of purified and specific cytoplasmic pollen extract: PureCyTonin®

Today, the extraction procedure for purified and specific cytoplasmic pollen extract under the trademark of PureCyTonin® (removed from its allergenic shell) enables retention of the active pollen components. PureCyTonin® is the main ingredient of the food supplement Serelys®, which is a combination of purified pollen/pistil cytoplasmic extracts. The pollen and pistils come from selected plants belonging to the Gramineae family and/or Pinaceae family and are harvested separately using a standardized method. Cultivation and harvesting take place in fields that are separated by plant type in accordance with the recommendations of the European Medicines Agency. The fields are free of pesticides and the chemical molecules usually used for plant growth and of any possible contamination. Manufacture of PureCyTonin® includes procedures that destroy the allergenic components of pollen. Allergens are broken down into peptides and amino acids. In the process, the pollen shells are completely removed, thus minimizing the risk of allergic reactions. The production procedures are standardized to ensure that batches of cytoplasmic pollen extract are reproducible. High-performance liquid chromatography (HPLC) and gas chromatography can verify that the group of compounds which forms the active substance is consistent between each batch. These extracts contain entities such as proteins, amino acids, sugar, minerals, vitamins, and fats. The amino acids, in particular, can be identified using analytical methods. Tryptophan, a precursor of serotonin, has been documented to be present in the pollen of the plants from which PureCyTonin® is extracted [11].

The method described makes it possible to exclude any allergen, thus ensuring that PureCyTonin® is safe to use. In addition, the exclusion of the shell, which constitutes a very stable protective pollen wall, makes the active compounds highly bioavailable [9,10]. Pollen extracts are water-soluble, and several compounds in the water-soluble pollen extracts are suitable. These include nutrients such as amino acids, carbohydrates, and minerals, as previously described [9,10]. However, no specific PK/PD data are available, and further studies should be performed. With a standardized extract blending procedure, 160 mg of PureCyTonin® and pollen/pistil extract is the main component of Serelys®, with a recommended daily dose of 320 mg. Serelys® has been used in Europe since at least 1995. A search on PubMed reveals a variety of commercial names: Serelys®, Femal®, Femalen®, Femelis Meno®, Menolesse®, Sansage®, and Relizen®. In France, it has received marketing authorization, under the name of Femelis, as a traditional herbal medicine used to relieve the symptoms of the menopause.

Preclinical studies with purified and specific cytoplasmic pollen extract: PureCyTonin®

PureCyTonin® has been evaluated in several preclinical studies, where it demonstrated its value as a safe and non-estrogenic alternative for menopausal symptoms (Table 1).

PureCyTonin® has been tested for acute oral toxicity. It was given to 10 mice at a single dose of 2000 mg/kg. The mice were followed for 2 weeks. All the animals survived, with no signs of toxicity, thus indicating that PureCyTonin® is very safe and non-toxic when given orally at a single high dose to these animals [12,13].

An OECD-approved genotoxicity test (the Ames test) has also been performed with PureCyTonin® to determine potential mutagenic activity. The Ames test uses bacteria to test whether a given product can cause mutations in the DNA of the test organism. It therefore serves as a quick and convenient assay to estimate the carcinogenic potential of a compound. The potential of PureCyTonin® for inducing reverse gene mutations has been examined, although no mutagenic activity was observed when the product was assessed using the Ames test [14,15]. A non-mutagenic extract is generally considered to have no carcinogenic potency and does not need to be further investigated for carcinogenesis.

Several *in vitro* and *in vivo* tests demonstrated that PureCyTonin® has no estrogenic action. A detailed study of the phytoestrogen composition by HPLC analysis revealed no trace of genistein, formononetin, or biochanin A, although it did show very minimal sub-effective concentrations of daidzein and genistein in these pollen extracts, thus ruling out any estrogenic action of pollen extracts.

Compared with ethinyl estradiol, PureCyTonin® did not show any uterotrophic effect in immature female rats when the uterotrophic screening assay was applied in juvenile Wistar rats [16]. No estrogenic effect was demonstrated in a reporter gene assay using human embryonic kidney 293T cells that were co-transfected with estrogen receptors ER alpha and ER beta and a luciferase reporter plasmid containing ER response elements. Compared with 17-b estradiol, PureCyTonin® was not able to induce transcriptional activation through the estrogen receptors [17].

The human breast cancer cell line MCF7 endogenously expresses estrogen receptors and has been widely used for the study of estrogenic compounds. These cells were used to test whether an estrogenic effect of pollen extract led to proliferation. PureCyTonin® did not show any effect on cell proliferation in MCF7 cells up to the highest concentration tested [17].

Table 1. *In vitro* and *in vivo* analysis with purified and specific cytoplasmic pollen extract: PureCyTonin®.

Type of study	Study	Publication/report	Type of measurement	Main results
No estrogenic activity	HPLC analysis	Hellstrom et al. (2012)	A detailed study of phytoestrogen composition	No trace of phytoestrogens
	Estrogenic activity with uterotrophic screening assay	Hellstrom et al. (2012)	Uterotrophic screening assay in juvenile Wistar rats	No uterotrophic effect *in vivo* compared with estradiol/no estrogenic activity
	Reporter gene assay in 293T cells. Cell proliferation in MCF7 cells	Espié M., IMS World Congress – Poster (2014) Espié (2013)	Transcriptional activation through the estrogen receptors ER α or ER β with an ERE-luc cotransfection Proliferation was determined by the MTT test	No affinity for estrogen receptors ER α and ER β/no estrogenic activity No proliferation on breast cancer cell line
	Proliferation and apoptosis MCF7 TD47 breast cancer cell line with PGRMC1 Comparison with estradiol (E2) and growth factors	Seeger et al. (2017)	MCF-7 and T47D cells were transfected with PGRMC1 Different concentrations of pollen extract alone and in combination with E2 or growth factor were tested Proliferation was determined using the MTT test Apoptosis was determined using the CDD ELISA kit	PureCyTonin® was neutral in the cell lines alone or in combination with E2 or growth factors in terms of cell proliferation and cell apoptosis, both in cells transfected with PGRMC1 and in cells not transfected with PGRMC1
	Potential interaction with tamoxifen	Goldstein et al. (2015)	Potential inhibition of CYPD26 enzyme at high concentrations in pooled liver microsome with quinidine as a control	No inhibition of CYPD26 with PureCyTonin® 6.53–10% compared with quinidine, which inhibits 100%
Potential mode of action	Selective serotonin reuptake inhibition, genomic study	Appel et al., GREM 2020, 1. in press.	Effects of PureCyTonin® on the uptake of [^{3}H]-serotonin into rat cortical synaptosomes RNA sequencing profile in SH-SY5 Y cells	Inhibition of the uptake of [^{3}H]-serotonin into rat cortical synaptosomes in a dose-dependent manner Neuropeptides and neurotransmitters genes are differentially modulated

Recently, PureCyTonin®, was analyzed to determine whether a small breast cancer risk may exist by stimulating steroidal receptors other than nuclear estrogen receptors, such as progesterone receptor membrane component (PGRMC1). Receptor membrane-initiated actions of PureCyTonin® were compared with estradiol (E2) and growth factors (IGF, FGF, EGF) in two different breast epithelial cancer cells (MCF7 and TD47) transfected with PGRMC1. The response of hormone receptor-positive breast cancer cells to estradiol (E2) and growth factors took the form of an increased proliferation rate and downregulation of apoptosis. PureCyTonin® was neutral in the cell lines transfected or not transfected with PGRMC1 in terms of cell proliferation and cell apoptosis compared to estradiol and growth factors. It was also neutral in combination with estradiol and growth factors in the two parameters tested. The authors conclude that the reduction in symptom scores reported from postmenopausal women treated with Serelys® may not be mediated by estrogen or estrogen-like pathways [18]. PureCyTonin® did not interfere with the efficacy of tamoxifen since it does not inhibit CYPD26, the enzyme which metabolizes tamoxifen to its active metabolite [19].

The data set out above support those reported by Espié [17] and recently by Biglia et al. [20] and Capozzi and Lello [21], who concluded that pollen extract is a non-estrogenic alternative for managing menopausal symptoms in cancer survivors.

Serelys® in menopause: clinical data

Serelys® has proven efficacious for the treatment of vasomotor symptoms in several clinical trials, including placebo-controlled trials, a clinical trial comparing treatment with MHT, and open-label studies (Table 2).

A randomized double-blind placebo-controlled study showed a significant reduction in hot flushes compared with placebo after 3 months. A total of 64 menopausal women were monitored during an initial phase of 1 month, during which their menopausal symptoms were recorded. This phase was followed by a 3-month period in which one group received Serelys® (at a rate of two tablets per day), while the other group received placebo. Sixty-five percent of women treated with Serelys® responded with a reduction in hot flushes compared with 38% in the placebo group ($p < .006$). In the Menopausal Rating Scale (MRS) evaluation, there was a 23% reduction in hot flushes in women taking Serelys® compared with placebo after 2 months. The reduction was 22% after 3 months. There was also an improvement in tiredness, dizziness, mood, libido, headache, irritability, mood swings, and sensitiveness in the Serelys® group compared with baseline ($p < .031$) [22].

In the same study, no differences were found in the hormone profiles of the two treatment groups, both of which had the expected high levels of FSH and low levels of estrogen. In addition, there were no changes in the level of SHBG or testosterone [22].

An open study conducted in France with 417 women taking Serelys® for 3 months showed an improvement or reduction in menopausal symptoms. The frequency of hot flushes was reduced by 65% (intensity by 64%), sweating and perspiration by 66% (intensity by 67%), irritability by 54%, and fatigue by 51%. Quality of life and tolerance were considered excellent in perimenopausal and menopausal women [23].

In a subanalysis, perimenopausal and menopausal women were assessed separately for tolerance and effectiveness of Serelys®. Serelys® proved to be very efficacious in many of the parameters analyzed, and tolerance was excellent in 92% of the patients [24]. In another study, standardized pollen extract was

Table 2. Clinical trials with Serelys® in menopause.

Type of clinical trial	Author and year of publication	Type of measurement	Main results
Randomized double-blind placebo-controlled	Winther et al. (2005)	64 women double blind placebo-controlled trial 3 months of treatment MRS, 15 Quality of life QoL parameters 15 QoL parameters Diary of AUB Blood samples for FSH, E2, TT, SHBG	65% of women responded, with a reduction in hot flushes compared with 38% in the placebo group ($p<.006$). 54 women were analyzed. The MRS evaluation revealed a 23% reduction in hot flushes with Serelys® compared with placebo after 2 months, and a 22% reduction after 3 months. Improvement in tiredness, dizziness, mood, libido, headache, irritability, mood swings, and sensitiveness) in the Serelys® group compared with baseline ($p<.031$) No changes in vaginal dryness parameter No AUB No change in blood levels of FSH, E2, TT, SHBG
Randomized placebo-controlled compared with MHT	D'Alterio et al. (2015)	45 women randomly treated either with Serelys® or with estrogen/progestin therapy or with placebo for 6 months. Evaluation at day 0, day 60, and day 90. The reduction in the Kupperman index was used for evaluation	Efficacy was slightly lower but similar to that of the estrogen/progestin therapy in peri and postmenopausal neurovegetative symptoms.
Open-label study	Elia et al. (2008)	417 women in France treated for 3 months, questionnaire and VAS evaluation at day 0 and day 90	The frequency of hot flushes was reduced by 65% (intensity by 64%), sweating and perspiration by 66% (intensity by 67%), irritability by 54%, and fatigue by 51%.
Open-label study	Druckman et al. (2015)	324 women. Analysis of efficacy and tolerance in perimenopausal and menopausal women. Evaluation at day 0 and day 90 through VAS.	Improvement in QoL by 53–72%. Reduction of intensity of menopausal symptoms in peri and menopausal women. No differences observed for either group.
Open-label Study	Paczkowski et al. (2018)	Quality of life in 50 women and reduction in hot flushes were assessed in an open study with the Menopause Rating Scale (MRS) and Female Sexual Functioning Inventory (FSFI) at 3 visits	Shows very good efficacy of the product with respect to elimination of vasomotor symptoms as early as after 4 weeks of treatment. This effect increased during the subsequent months of treatment.
Open-label study	Fait et al. (2019)	104 women in the Czech Republic treated for 3 months and evaluated with the Menopause Rating Scale (MRS) at day 0, day 30, day 60, and day 90	Significant decrease in menopausal symptoms between the starting point of the study and after 12 weeks ($p<.0001$). Hot flushes were reduced by 48.5%, sleep disturbances by 50.1%, and depressive mood by 51.2%.

associated with an improvement in perimenopausal symptoms such as hot flushes and mood swings [25].

D'Alterio et al. [26] showed benefits for peri- and post-menopausal neurovegetative symptoms in a clinical trial where women were treated with Serelys®, estrogen/progestin therapy, or placebo for 6 months. This study confirmed efficacy in reducing the intensity of the neurovegetative symptoms of menopause. The reduction in the Kupperman index was slightly lower, but similar to that of estrogen/progestin therapy.

Quality of life and reduction in hot flushes were recently assessed in Poland in an open-label study that demonstrated efficacy with respect to the resolution of vasomotor symptoms after as few as 4 weeks of treatment; this effect increased during the subsequent months as well. The benefits of Serelys® in perimenopausal women are multidirectional in nature and pertain to many somatic and nonsomatic elements responsible for the quality of life [27].

A prospective observational study to evaluate the efficacy and safety of Serelys® in the management of women with menopausal symptoms was performed in 104 women in the Czech Republic. This study showed a significant decrease in various menopausal symptoms from baseline through 12 weeks ($p < .0001$). Hot flushes were reduced by 48.5%, sleep disturbances by 50.1%, depressive mood by 51.2%, irritability by 47.9%, and fatigue by 47.8%. Menopausal symptoms were reduced significantly, with very few side effects [28].

Taken together, these data show that PureCyTonin® is an effective non-hormonal, non-pharmacological therapy for the management of hot flushes and menopausal symptoms. Compared with non-pharmacological interventions and other non-hormonal treatments, it is effective and safe in women who do not wish to take MHT and women for whom MHT is contraindicated, e.g. women with breast cancer who turn to natural supplements and remedies for relief.

In the 2015 position statement of the North American Menopause Society on non-hormonal management of menopause-associated vasomotor symptoms, pollen extract was already classified as 'Over-the-counter supplements and herbal therapies'. However, in this position statement, only one randomized controlled trial in menopause was evaluated [6,22]. In France, marketing authorization has been granted under the name Femelis as a traditional herbal medicine used to relieve the symptoms of menopause.

Although PureCyTonin® is not yet universally approved according to the main guidelines, the studies documented in this review point to PureCyTonin® as a non-estrogenic alternative for managing menopausal symptoms. It can also be administered in cancer survivors. The purpose of this review is to improve the

quality of care by generating and disseminating information on pollen extract that can help to diversify professional practices. Therefore, PureCyTonin® and the finished product, Serelys®, could be recommended in the guidelines of the main gynecological societies as a non-hormonal alternative for the management of menopausal symptoms and to improve patients' quality of life.

Clinical data on Serelys® in premenstrual symptoms

Premenstrual syndrome often includes symptoms such as anxiety, irritability, depression, loss of confidence, mood swings, and fatigue. There are also physical symptoms, typically bloating, water retention, and breast pain. It is the timing, rather than the types of symptoms, and the degree of impact on daily activity that supports a diagnosis of PMS. The symptoms, which may severely affect quality of life, occur during the 2 weeks before menses, and some signs continue during menstruation before disappearing a few days afterwards [29]. These manifestations can occur in women from their late 20s to their early 40s and have a negative impact on their professional, social, and sexual lives. PMS is caused by changes in normal hormone levels that can induce disorders in the central neuroendocrine system. It is well established that serotonin plays a role in PMS, as some selective serotonin reuptake inhibitors (SSRIs) have a beneficial impact on premenstrual disorders. A meta-analysis of all available randomized controlled trials involving SSRIs used in PMS confirmed these to be more efficacious than placebo [30–32].

PureCyTonin® has been proven to be beneficial for the management of PMS in various randomized, double-blind, placebo-controlled clinical trials (Table 3). One randomized double-blind placebo-controlled cross-over trial showed the efficacy of Serelys® in women with PMS. The study included 32 women. Group A received two Serelys® tablets twice daily for two consecutive cycles, whereas group B received placebo for two cycles. Groups A and B were then crossed over: those taking the placebo took the active product, and vice versa. Two months of active treatment lowered overall symptom indices significantly and lowered six of nine individual symptom scores by 27–57%. Symptoms (e.g. irritability, swelling, weight fluctuation, and mood swings) improved significantly in women with PMS when they took the active product ($p < .05$). Evidence for a slow onset of action and protracted effect was provided by the finding that all symptom indices studied declined significantly (by 48–88%) in the group that received placebo before active treatment ($p < .01$). Premenstrual weight gain was reduced by 50% in patients receiving active treatment compared with placebo. There were no reported unwanted or adverse effects during active treatment [33].

Another randomized double-blind placebo-controlled multicenter trial looked at the efficacy of Serelys® in PMS: 50 women taking two tablets of the active product per day were compared with 51 women on placebo. The study duration was four cycles. Compared with placebo, Serelys® was associated with a significant improvement in PMS symptoms, particularly in sleep disturbances. This reduction was further observed in women who reported irritability as their major PMS symptom ($p < .001$). There were no differences in adverse events in the active treatment group compared with placebo [34].

Winther et al. [35] confirmed these results in a recent subanalysis in which women with PMS symptoms were separated depending on whether the predominant symptom was irritability or dysphoria. The authors showed that in patients reporting irritability as their main PMS symptom, Serelys® significantly lowered PMS indicators, as reflected in global scores in both observer-based and self-evaluation scoring of symptoms.

Mechanism of action of Serelys®

During the menopause transition, neuronal cells, neurotransmitters, neuropeptides, and neurosteroids in the central nervous system (CNS) undergo important changes as a consequence of fluctuations in the levels of hormones such as estrogen [36]. These changes could be responsible for the generation of hot flushes and modifications in behavior, cognition, and mood. There is evidence of a neuroendocrine origin and a key role of neurotransmitters (e.g. glutamate, gamma-aminobutyric acid (GABA), serotonin (5-hydroxytryptamine [5-HT]), dopamine, acetylcholine noradrenaline), since some of the known treatments for hot flushes and modifications in behavior, cognition, and mood are compounds that modulate these neurotransmitters [37,38]. SSRIs are used for their known effect on thermoregulation and sleep quality, by acting on serotonergic neurons. Recently, a specific type of neurons – KNDy kisspeptin/neurokinin B/dynorphin neurons – have been shown to be present in the hypothalamus at the level of the preoptic area. These change dramatically in postmenopausal women, in whom they can generate hot flushes [39]. In this subpopulation of neurons, in particular, neurokinin B and its receptor NK3R have been implicated in the induction of hot flushes in healthy women that are typical in location and duration of postmenopausal hot flushes [40,41]. In order to gain more insights into the mechanism of action of Serelys® on neurons, the effect of PureCyTonin® on the uptake of [^{3}H]-serotonin into rat cortical

Table 3. Clinical trials with Serelys® in premenstrual symptoms.

Type of clinical trial	Author and year of publication	Type of measurement	Main results
Randomized double-blind placebo-controlled	Winther et al. (2002)	32 women treated either with Serelys® for 2 consecutive cycles or placebo for 2 consecutive cycles. Groups A and B were then swapped over: those taking the placebo took the active product and vice versa	Efficacy in women with premenstrual syndrome irritability, swelling, weight fluctuation, mood swings, etc. were significantlyreduced ($p < .05$).
Randomized double-blind placebo-controlled	Gerhardsen et al. (2008)	Multicenter study with 101 women treated either with Serelys® or placebo for 4 cycles.	This improvement was observed mainly for the 'irritability' symptom ($p < .001$).
Randomized double-blind placebo-controlled	Winther et al. (2018)	Sub-analysis of 101 women in 2 groups: one reported irritability as their main PMS symptom the other reported dysphoria as their main PMS symptom	Daily intake of 30 mg of Serelys® for 4 cycles led to a 50% reduction in various PMS symptoms in patients with irritability as their main symptom. The reduction was versus placebo. Such a reduction was not seen in the dysphoria group.

synaptosomes was analyzed. PureCyTonin® inhibited the uptake of [^{3}H]-serotonin into rat cortical synaptosomes in a dose-dependent manner. The EC_{50} value of 60 µg/ml is well within a physiologically achievable order of magnitude. Inhibition of serotonin uptake may therefore play a role in the physiological action of Serelys® [42]. PureCyTonin® could act as an SSRI-like therapy, potentially without the known adverse effects of SSRIs. Additionally, tryptophan, a precursor of serotonin, is present in the pollen of the plants from which PureCyTonin® is extracted [11]. The average level of tryptophan in pollen extracts measured in Serelys tablets is about 0.09 mg/tablet [43]. Taking these observations together, pollen extract appears to maintain the availability of serotonin in hypothalamic serotonergic neurons, and this could explain in part the efficacy of the extract in controlling thermoregulation, sleep, and mood in menopausal women. There are also dopaminergic neurons located in the frontal cortex, the nucleus accumbens, the hippocampus, and the striatum. These dopaminergic neurons are responsible for pleasure, mood, motivation, and perseverance [44]. Decreased levels of estradiol in postmenopausal women may reduce dopaminergic neurotransmission and impair cognitive and motor functions [45].

A genomic study was performed in neurons to further investigate which pathways are modulated by PureCyTonin® and better understand its correlation with beneficial biological effects [42]. The human cell line SH-SY5Y, which comprises cells differentiated by all-trans-retinoic acid (RA), has long been used to study neuronal signaling pathways modulated by chemical entities. RA treatment induces differentiation of these proliferating cells to cells with a cholinergic neuron phenotype exhibiting neuron-like properties, including neurite, and expressing cholinergic neuronal markers. In this study, the cells were treated with or without PureCyTonin®, and mRNA extracted from SH-SY5Y cells was analyzed with mRNA-seq profiling. Significantly modulated genes underwent pathway analysis using the software application Ingenuity Pathway Analysis (Qiagen, Germantown, MD) to identify pathways of interest that could be modified by pollen extract. Preliminary results show several modulated genes that may correlate with the beneficial effects induced by PureCyTonin® in menopausal women. These include the dopamine D2 receptor (DRD2), which was significantly modulated [42]. DRD2 is implicated in several domains, including motivation, pleasure, cognition, memory, learning, and fine motor control, as well as in modulation of neuroendocrine signaling. Interestingly, it has been shown that estradiol activates the transcription of DRD2 in the frontal cortex and increases the level of DRD2 mRNAs. DRD2 declines substantially during a person's lifetime, and animal studies have shown age-related reductions in dopamine release and dopamine markers in the cerebral cortex [46]. Decreased levels of estradiol in post-menopausal women may reduce the expression of D2 receptors, thus contributing to reduced dopaminergic neurotransmission and impairment of cognitive and motor functions. Hence, this increased expression in DRD2 induced by pollen extract may benefit menopausal women. Current analysis is focusing on other genes coding for neuropeptides implicated in nociceptive pain, cognition, feeding, and various regulatory processes (waking and sleep, mood, blood pressure, osmosis, and water intake). In one study, pollen extract increased the expression of protective enzymes such as NQO1, an antioxidant enzyme that exerts its effect by scavenging superoxide to shield against oxidative stress actuated by cytotoxic substances. This action could provide additional cytoprotection and prove especially effective in tissues with low levels of superoxide dismutase expression in pro-oxidant environments. It has been shown that the marked reduction in estrogen during menopause increases levels of oxidative stress in the body [47]. Part of the mechanism of action of PureCyTonin® could also be explained by its antioxidant activity. Compared with other approaches, pollen extracts have been shown to exhibit superoxide dismutase activity in the generation of oxygen free radicals [48].

Conclusions

Serelys® is an effective and safe non-hormonal alternative for alleviating menopausal symptoms without increasing the risk of breast cancer in women in whom MHT is contraindicated or who do not wish to use hormones. Serelys® has proven to be safe and efficacious. It improves hot flushes, sleep disturbances, and nervousness in menopausal women. The beneficial effects are also observed in PMS. These findings seem to correlate with the effect of its main ingredient, PureCyTonin®, on inhibition of the reuptake of serotonin and other neurotransmitters. Current studies are being performed to fully determine the mode of action of PureCyTonin®.

Disclosure statement

Andrea Genazzani: Consultant and occasional presentations for Abbot, Alfa Sigma, Bionorica, Endoceutics, Exeltis, Grunenthal, Mithra, MSD, Se-Cure, Pharma, Serelys Pharma, IBSA, Theramex.

Nick Panay: Speaker's bureau: Abbott, Bayer, Besins, Meda, MSD, Mylan, Novo Nordisk, SeCur, Shionogi, Theramex. Advisory Committee: Abbott, Bayer, Besins, Meda, Mithra, MSD, Mylan, Novo Nordisk, Kora, SeCur, Shionogi. RESEARCH GRANTS: Abbott, Asarina, Mithra, Mylan, NeRRe, Pharm Olam, Yes Company.

Tommaso Simoncini, Herman Depypere, Christian Egarter, Nicoletta Biglia, Tomas Fait, Martin Birkhaeuser, Sven O. Skouby, Mark Brincat, Cuauhtémoc Celis-Gonzales: No potential conflicts of interest were reported by the authors.

Steven Goldstein: Gyn Advisory Boards for AbbVie, Amag, Amgen, Astellas, Therapeutics MD.

Xiangyan Ruan: Experimental research on Purified and Specific Cytoplasmic Pollen Extract was financially supported by Serelys Pharma.

Santiago Palacios: Symposium speaker or advisory board member for Shionogi, Pfizer, Lacer, Theramex, Procare, Serelys Pharma, and Amgen. Received research grants and/or consulting fees from Pfizer, Serelys Pharma, Amgen, MSD, Seid, lacer, Leon Farma, Gynea, and Sandoz.

ORCID

Tommaso Simoncini http://orcid.org/0000-0002-2971-0079
Nicoletta Biglia http://orcid.org/0000-0003-1009-5309

References

[1] Santoro N, Randolph JF. Reproductive hormones and the menopause transition. Obstet Gynecol Clin North Am. 2011;38(3):455–466.
[2] Davis S, Lambrinoudaki I, Lumsden M, et al. Menopause. Nat Rev Dis Primers. 2015;1(1):15004.
[3] Freedman RR. Menopausal hot flashes: mechanisms, endocrinology, treatment. J Steroid Biochem Mol Biol. 2014;142:115–120.

[4] Depypere HT, Comhaire FH. Herbal preparations for the menopause: beyond isoflavones and black cohosh. Maturitas. 2014;77(2):191–194.
[5] Mintziori G, Lambrinoudaki I, Goulis DG, et al. EMAS position statement: non-hormonal management of menopausal vasomotor symptoms. Maturitas. 2015; 81(3):410–413.
[6] Non hormonal management of menopause-associated vasomotor symptoms: 2015 position statement of The North American Menopause Society. Menopause. 2015;11:1155–1174.
[7] Stanley RG, Linskens HF. Pollen biology biochemistry management. Berlin, Heidelberg, New York: Springer; 1974.
[8] Komosinska-Vassev K, Olczyk P, Kaźmierczak J, et al. Bee pollen: chemical composition and therapeutic application. Evid Based Complement Alternat Med. 2015;2015:1–6.
[9] Bogdanov S. Pollen: production, nutrition and health: a review. Bee Product Science; 2014 [cited 2020 Jan 29]. Available from: http://www.bee-hexagon.net/
[10] Rimpler M. Von Bienen gesammelte Blutenpollen: Eigen-schaften und Verwendung. Arztezeitschrift Naturheilver-Fahren. 2003;3: 158–165.
[11] Goss JA. Development, physiology, and biochemistry of corn and wheat pollen. Bot Rev. 1968;34:333–359.
[12] Bollen LS. Polbax extract. Acute toxicity study in the rat Scantox test report Lab N° 25550 29.09; 1997.
[13] Jensen D. Romert. Limited test for acute oral toxicity in mice of Royal Sport Extract. Genotox project N°114-001 N° F11-005; 1988.
[14] Kraft M. Reverse mutation assay using bacteria (*Salmonella typhimurium*) with pollen extract GCFem. Bioservice Scientific Laboratories Project 072759 BSL Bioservice report 072759; 2007.
[15] Kraft M. Reverse mutation assay using bacteria (*Salmonella typhimurium*) with polbax pollen extract. Bioservice Scientific Laboratories Project 072760 BSL Bioservice report 072760; 2007.
[16] Hellström AC, Muntzing J. The pollen extract femal – a non-estrogenic alternative to hormone therapy in women with menopausal symptoms. Menopause. 2012;19(7):825–829.
[17] Espié M. Bouffées de chaleur et cancer du sein: quelle prise en charge efficace et sans risque? Cancer Féminin. 2013;III:1–16.
[18] Seeger H, Ruan X, Neubauer H, et al. Membrane-initiated effects of Serelys® on proliferation and apoptosis of human breast cancer cells. Gynecol Endocrinol. 2017;34:1–4.
[19] Goldstein S, Espie M, Druckmann R. Does purified Swedish pollen extract, a nonhormonal treatment for vasomotor symptoms, inhibit the CYP2D6 enzyme system? Menopause. 2015;11:1212–1214.
[20] Biglia N, Bounos VE, De Seta F, et al. Non-hormonal strategies for managing menopausal symptoms in cancer survivors: an update. Ecancer. 2019;13:909.
[21] Capozzi A, Lello S. Purified pollen extract and vasomotor symptoms management. GIOG. 2018;40(1):7–9.
[22] Winther K, Rein E, Hedman C. Femal®, a herbal remedy made from pollen extracts, reduces hot flushes and improves quality of life in menopausal women: a randomized, placebo-controlled, parallel study. Climateric. 2005;8(2):162–170.
[23] Elia D, Mares P. Evaluation de la tolérance et de l'efficacité d'un complément alimentaire Sérélys® (Femal®) chez les femmes en période de ménopause. Génésis. 2008;135:12–15.
[24] Druckmann R, Lachowsky M, Elia D. Evaluation de la qualité de vie, de l'efficacité et de la tolérance de Sérélys chez les femmes en période de péri-ménopause et ménopause. Genesis. 2015;183:10–13.
[25] Kimura H, Gruber P. Perimenopausal symptoms such as hot flushes and mood swings are reduced by a standardised pollen pistil extract. Climacteric. 2002;5(Suppl. 1):85.
[26] D'Alterio MN, Giancane E, Cornacchia S, et al. GCFem, PI82, vitamin E in menopause treatment: benefits for peri and postmenopausal neurovegetative symptoms. Multidisc J Women's Health. 2015;4(1).
[27] Paszkowski T, Skrzypulec-Plinta V. Assessment of quality of life in women using Femelis Meno. Prz Menopauzalny. 2018;17(2):77–85.
[28] Fait T, Sailer M, Regidor PA. Prospective observational study to evaluate the efficacy and safety of the pollen extract Sérélys® in the management of women with menopausal symptoms. Gynecol Endocrinol. 2019;35(4):360–363.
[29] Van Keep PA, Lehert P. Le syndrome prémenstruel; une étude épidémiologique en France. Contracept Fertil Steril. 1980;8:775.
[30] Dimmock P, Wyatt KM, Jones PW, et al. Efficacy of selective serotonin reuptake inhibitors in premenstrual syndrome: a systematic review. Lancet. 2000;356:1131–1136.
[31] Panay N. Management of premenstrual syndrome. J Fam Plann Reprod Health Care. 2009;35(3):187–194.
[32] Panay N, Studd J. The management of PMDD through ovarian cycle suppression. In: O'Brien S, Rapkin AJ, Schmidt PJ, editors. Premenstrual syndromes: PMS and PMDD. Informa UK; 2007. p. 121–130.
[33] Winther K, Hedman C. Assessment of the effects of the herbal remedy Femal on the symptoms of premenstrual syndrome a randomized, double blind, placebo-controlled study. Curr Ther Res. 2002;5: 344–353.
[34] Gerhardsen G, Hansen AV, Killi M, et al. The efficacy of Femal® in women with premenstrual syndrome: a randomised, double-blind, parallel-group, placebo-controlled, multicentre study. Adv Ther. 2008; 25(6):595–607.
[35] Winther K, Campbell-Tofte J, Motawei AM, et al. A double blinded, randomized, placebo controlled, parallel study of pollen pistil extract (Serelys) on women reporting irritability as predominant PMS symptoms. J Herb Med. 2018;12:23–32.
[36] Giannini A, Caretto M, Genazzani A, et al. Optimizing quality of life through sex steroids by their effects on neurotransmitters. Climacteric. 2019;1:55–59.
[37] Rossmanith WG, Ruebberdt W. What causes hot flushes? The neuroendocrine origin of vasomotor symptoms in the menopause. Gynecol Endocrinol. 2009;25(5):303–314.
[38] Berendsen HH. The role of serotonin in hot flushes. Maturitas. 2000; 3:155–164.
[39] Rance NE, Dacks PA, Mittelman-Smith MA, et al. Modulation of body temperature and LH secretion by hypothalamic KNDy (kisspeptin, neurokinin B and dynorphin) neurons: a novel hypothesis on the mechanism of hot flushes. Front Neuroendocrinol. 2013;3:211–227.
[40] Prague JK, Roberts RE, Alexander AN, et al. Neurokinin 3 receptor antagonism as a novel treatment for menopausal hot flushes: a phase 2, randomised, double-blind, placebo-controlled trial. Lancet. 2017; 10081:1809–1820.
[41] Depypere H, Timmerman D, Donders G, et al. Treatment of menopausal vasomotor symptoms with fezolinetant, a neurokinin 3 receptor antagonist: a phase 2a trial. J Clin Endocrinol Metabol. 2019;12: 5893–5905.
[42] Appel K, Veit J, Diaz Ph, et al. Purified and specific cytoplasmic pollen extract, PureCyTonin®, a non-hormonal treatment for hot flushes, inhibits serotonin reuptake. Gynecol Reprod Endocrinol Metabol.; 2020;1; in press.
[43] Svensson U. Report EUDAKG Eurofins Steins Laboratorium. Method EU 152/2009. AR-16-LW-035803-012016; 2009.
[44] Berridge KC, Kringelbach ML. Pleasure systems in the brain. Neuron. 2015;3:646–664.
[45] Zichella L, Falaschi P, Fioretti P, et al. Effects of different dopamine agonists and antagonists on post-menopausal hot flushes. Maturitas. 1986;3:229–237.
[46] Sárvári M, Kalló I, Hrabovszky E, et al. Estradiol replacement alters expression of genes related to neurotransmission and immune surveillance in the frontal cortex of middle-aged, ovariectomized rats. Endocrinology. 2010;151(8):3847–3862.
[47] Doshi SB, Agarwal A. The role of oxidative stress in menopause. J Midlife Health. 2013;4(3):140–146.
[48] Krotkiewski M, Belboul A, Palm S, et al. The effect of SOD-active plant substance Polbax on oxygen free radical (OFR) generation and blood cell rheology. Clin Hemorheol. 1995;4:641–647.