

Vitex agnus-castus

and the way of the drug to the clinical approved herbal medicinal product.

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Vitex agnus-castus has a long tradition as a herbal remedy and was used in ancient times not only as an aphrodisiac and for preserving chastity but also against diverse disturbances of the female genital system. In actual clinical trials, the fruit *Agni casti fructus* was shown to relieve premenstrual syndrome (PMS) and especially breast swelling and pain, due to its dopaminergic effect.

Although approximately 50% or more of young women suffer from PMS, there is no generally accepted therapy and only a few preparations for that purpose are available on the Swiss market. Therefore the extract preparation Ze440 was developed and its effectiveness has been studied in a clinical trial. In a controlled, double blind study 170 women suffering from PMS were treated with Ze440 during three menstrual cycles. 52% of the treated patients had a significant improvement in the combined symptoms, relative to those on placebo (24%). The positive results of the clinical trial show that PMS can successfully be treated with the extract preparation Ze440. Today the product is available in the Swiss market under the brand names Prefemin® and Premens®.

Botanical description

Vitex agnus-castus L., the chaste tree, is a shrub belonging to the genus *Vitex* of the *Verbenaceae* family. It is widespread on riverbanks and on shores in the Mediterranean region and in Asia. *V. agnus-castus* is a deciduous shrub which reaches heights of up to 5 m. The leaves are opposite, hand-shaped, composed of five to seven radiating leaflets which are borne on a main stalk. The leaflets are linear, lance-shaped, toothed, dark green above and grey beneath with a very close felt. In August to October flower panicles with numerous flowers are formed. The flowers are fragrant and of blue, lilac, rose or white colour. The berries resemble peppercorns, hard, with a purple to black skin, yellowish within, half-covered by their sage-green calyces and containing four seeds. The odour is aromatic and spicy, the taste warm and peculiar. The parts used today as herbal medicine are the dried ripe berries (*Agni casti fructus*) [1].

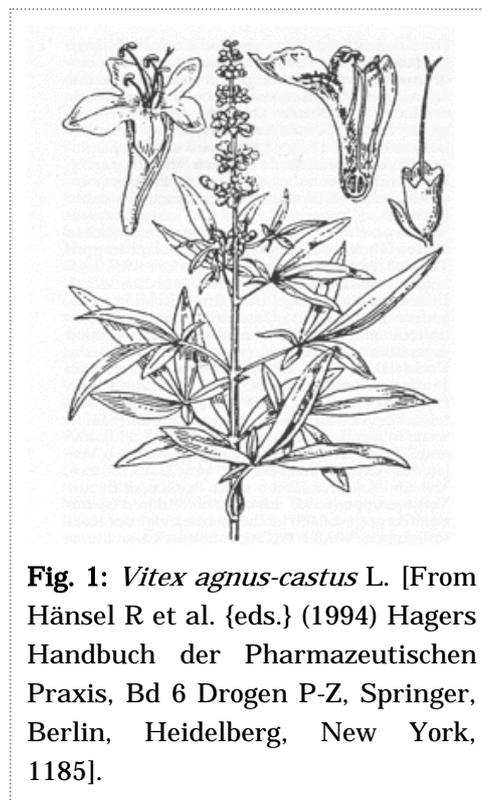


Fig. 1: *Vitex agnus-castus* L. [From Hänsel R et al. {eds.} (1994) Hagers Handbuch der Pharmazeutischen Praxis, Bd 6 Drogen P-Z, Springer, Berlin, Heidelberg, New York, 1185].

Historical aspects and traditional use

The plant *V. agnus-castus* has been used medicinally for at least two thousand years. The Greek goddess Hera is said to have been born under an *Agnus-castus* shrub. The shrub was a symbol of chastity in those days. Women of ancient Athens, who celebrated the thesmophory, a festival for the goddess Demeter, adorned themselves with *V. agnus-castus* flowers and spread the leaves on their couches for preserve chastity. In the middle-ages, Christian monks used the berries, which smell

and taste like pepper, as a spice and as an aphrodisiac, hence the derivation of the name 'monk's pepper'. In medieval monasteries the monks still used to spread the herb on their couches for chastity. Dioscorides, a Greek physician, mentioned it as a beverage taken to lower libido. Even at that time the plant was namely used for 'dysregulations of the female genital system'. In addition, the fruit was said to be useful not only to bring on menstruation and to relieve uterine cramps but was also used as a lactogen, as an anorectic, as a hypnotic and for dyspepsia [2, 3].

Constituents

Agni casti fructus belongs to the drugs, in which until now no substance is known, to which the efficacy could be attributed clearly. The extract consists of a multitude of constituents, of which none reaches a share of >1%. Although an HPLC-fingerprint shows many peaks, only few substances are known until now. In many cases casticin, a lipophil flavonol, is dominant. In the drugs, which are rich in casticin, contents of 0.1-0.2% were determined [4]. Casticin is accompanied by some other flavonols, which are, however, found to a far lower extent. Flavonol-C-glycosides, are only found in low amounts as compared to hawthorn leaves with flowers and passion flower herb. Isoorientin can be detected in TLC as well as in HPLC-fingerprint in all samples. The iridoid glycosides agnusid is a good marker for *Vitex agnus-castus*, since the substance is less frequent in other species than aucubin, which is also to be found.

Furthermore, 4-hydroxybenzoic acid is detected in the fingerprint chromatogram. This substance is ubiquitous. Three new diterpenoides (vitexilactone, rotundifurane, 6 β ,7 β -diacetoxy-13-hydroxy-labda-8,14-diene) could be isolated and elucidated in their chemical structure [5]. HPLC-analyses showed amounts of 0,3-0,5% of diterpenoids in dried fruits rich in casticin (>0,15%). Naturally, the drug contains fatty oils, including linoleic acid [6]. The volatile oil of the fruits of *Vitex agnus-castus* was examined several times, but it is of low importance in a dry extract preparation.

An assessment of the results of Saden et al. [7], who postulated hormones such as progesterone, testosterone, and epitestosterone in *Vitex agnus-castus* did not lead to positive results. By means of different procedures (TLC, HPLC) these compounds could not be detected in the fruits [4].

The way of the drug to the clinical approved herbal medicinal product.

Introduction

A study realised at the University of Basle [8] showed that the prevalence of the PMS in young women is surprisingly high. 69.2% of 132 women interviewed suffer under PMS symptoms. Quality of life is negatively influenced by PMS in more than 50% of the women, who observed symptoms between one and ten days before the beginning of the menstruation. Furthermore in a telephone inquiry of 1000 women at the age of 15-74 years (Random-Quota-procedure, unpublished data) in the German and French speaking part of Switzerland, 56.3% of the questioned women answered the question 'do you suffer from PMS-complaints' with no, 20.1% however with yes, often and 23.7% with yes, sometimes. 10.9 % of the 292 questioned women with PMS-symptoms felt very restricted in their well-being. 32.5% of them treat their complaints, mainly with analgesics (43,2%). Thus, the need for a medicament to manage premenstrual complaints has been shown.

A clinically documented and generally accepted therapy for the PMS has not been available until now. The recommended therapies are based on medical and personal experience. Regarding this, chaste-tree extracts belong to the best documented therapy forms. The number of preparations available for that purpose is, especially in Switzerland, very low. In order to bridge this gap, the extract preparation Ze440 was developed. In order to improve the knowledge of *Agni casti fructus*, parallel to the drug development a phytochemical dissertation at the ETH Zurich and a pharmacological-clinical dissertation at the University of Basle were initiated and meanwhile

finalised. The project is an impressive example of a successful co-operation between industry and university.

Galenic development of extract preparation Ze440 and its solid dosage form Z-94040:

a) Extract:

The extract development proceeded from the monograph of the Commission E, which recommends aqueous-alcoholic extracts (50-70%) from the crushed fruits. With two batches of dried drug the extract yield was tested. 60% (m/m) ethanol has been evaluated as the optimised solvent for the extraction of casticin and agnusid and for a high yield of extractive substances.

b) Standardisation:

The fact that no substance, which determines the efficacy can be defined in the chaste tree extracts permits no so-called standardisation on a certain constituent. Correspondingly 20 mg of the native extract is defined as the active constituent. Since in pharmacological tests the activities were clearly found in the lipophil fractions casticin was chosen as a marker for the batch-specific control. In order to be able to comprehend the analytics adequately, a minimum value (based on present experience) for casticin in the drug (0.08%) and in the extract (0.6%) had to be specified.

Fingerprint analysis of the drug shows remarkable quantitative differences of casticin, isoorientin and agnusid. Drugs will be primarily selected to produce the extract Ze440 when all mentioned peak groups have been detected in balanced amounts.

The standardisation includes the validated extraction procedure. It is based on the carefully selected drug with a high yield of extractives and a specific phytochemical character as described.

c) Extract dosage:

In empirical medicine there are only few data on the dosage of *Agni casti fructus*. For Z-94040 the dosage of 20 mg of native extract per tablet, already registered in Switzerland, was chosen and was maintained because of the positive experience in the clinical trial [8]. With a drug/extract ratio (DER) of approximately 6-12:1 the 20 mg native extract corresponds to 120-240 mg of dried drug.

d) Batch to batch reproducibility

The standardisation concept has been evaluated retrospectively by HPLC and TLC analyses. The TLC-fingerprints of diterpenoides and casticin of all the extracts produced up to now show similar chromatograms concerning spots and their intensity [9]. Quantitative HPLC-analyses showed a complete diterpenoid characteristic with the three isolated compounds within a range of 2-4% [6]. That means: The standardisation concept selected for the product guarantees the batch to batch reproducibility in a convincing way. Furthermore, this reproducibility has been checked and approved on a pharmacological level as well (see below).

e) Solid dosage form

Since *Agni casti* extracts require long-term treatment, with a daily intake on a long-term basis, tablets are most suitable dosage form for that purpose. Therefore a film-coated tablet was chosen as administrative form for Z-94040. The dry extract is mixed with the excipients necessary for the direct tableting and is compressed on a rotary tableting machine. Special disintegrating agents were not necessary, since the small tablets (diameter 8 mm) disintegrate quite quickly. The coating is realised in a suitable coater with hydroxypropylmethyl-cellulose, pigmented white with titanium dioxide. Only excipients monographed in the Ph Eur are used.

Pharmacology

Based on the positive screening results obtained with the concentrated extract V23/95 at the beginning of the project and the findings of earlier research, the pharmacological experiments have been mainly focused onto the binding on the dopamine D₂ receptor sites. The comparison of five batches of the dry extract preparation Ze440 on the inhibition of the binding of ³H-spiperone to the dopamine D₂ receptor showed similar inhibition potencies with IC₅₀ values between 40 and 70 µg/mL. The chemical results as well as the pharmacological experiences support the standardisation concept of the extract. No loss of activity has been observed after the drying process: The concentrated extract and the dry extract preparation of batch V23/95 showed the same activity.

In order to identify the diterpenes exhibiting inhibitory binding effects to D₂ receptors, dried fruits were directly extracted with hexane and studied. This extract potently inhibited D₂ binding with an IC₅₀ value of 15 µg/mL. Three diterpenes have been isolated. Two of these exhibited inhibitory actions with IC₅₀ values of 194 (6β,7β-diacetoxy-13-hydroxy-labda-8,14-diene) and 124 (rotundifuran) nmol/mL. In addition, linoleic acid showed a similar potent binding inhibition as the diterpenes, whereas other characteristic constituents of *V. agnus-castus* such as agnuside, aucubin, casticin and other flavonoids did not show any inhibitory effect at all.

In former examinations, dopaminergic constituents were suspected in the hydrophilous fraction of alcoholic extracts. This could be disproved by the presented studies. Interestingly, the IC₅₀-values of the pure substances did not exceed those of the full extract [8].

No significant inhibition binding of the extract to histamine H₁, benzodiazepine and OFQ receptor nor to the 5-HT transporter have been found. However, inhibition of binding to the µ, κ and δ opioid receptor with IC₅₀ values of 36, 22 and 194 µg/mL, respectively was observed. Interestingly, activity was not exclusively observed in the hexane fraction as it was the case for D₂ receptors, but in all subfractions (aqueous, butanol, chloroform and hexane fractions).

In order to determine the stability of the extract, the experiments on µ and κ receptors were repeated one year later. Similar results (15 and 20 µg/mL for µ and κ-receptor) were obtained, confirming the pharmacological stability of the extract. More details in [10].

Clinics

In phytotherapy, convincing results in chemistry and pharmacology are the base to guarantee a constant efficacy of the extracts, but the medication has to be approved. Therefore, to prepare the launch of the product, a prospective, multicentre trial with Ze440 has been started. 50 women, suffering from PMS have been included in the study. The patients were treated daily with one tablet during three menstrual cycles. 43 patients completed the whole study protocol which includes 8 menstrual cycles, i.e. 2 baseline, 3 treatment and 3 post-treatment cycles. 30 patients without and another 13 with oral contraceptives took part in the study. 6 patients were drop-outs without any relation to the study medication, one patient complained of fatigue and headache which might be related to study medication. All evaluated patients took at least 85% of the medication. The main effect parameter was the validated Moos' menstrual distress questionnaire (MMDQ), secondary parameters were a visual analogue scale (VAS; self-assessment) and a global impression scale (GI, self-assessment).

The PMS related symptoms were reduced by treatment. The severity of symptoms returned after treatment's cessation. However, a difference to baseline remained. 23 of the included 43 patients are considered clinically relevant responder, because their MMDQ score dropped ≥ 50% of the baseline value.

At baseline, the VAS score was elevated in the late luteal phase and low at the follicular phase, as expected. By treatment, in the late luteal phase, the elevated VAS score became reduced (47.2%; $p < 0.001$) and returned to 21.7% ($p < 0.001$) under baseline at the end of the post-treatment period.

The low VAS score within the follicular phase remained unchanged over the whole observation period. 38 patients judged the global efficacy from moderate to excellent, 5 patients indicated no global efficacy. The number of days patients suffered from PMS slightly reduced from 7.5 to 6. No differences were seen between patients with or without oral contraceptives.

No serious adverse event occurred. A patient dropped out after four days of treatment because fatigue and headache. The safety control parameters did not change critically at all, incidental differences remained within limited physiological ranges. More details in Berger (1999) [11].

The results of the so-called "Berger-study" stimulated to start the first randomised, double-blind, placebo-controlled, parallel group PMS-study with an extract of the fruits of *Vitex agnus-castus*. The dose was one tablet with 20 mg of Ze440 daily (or matching placebo), given during 3 consecutive menstrual cycles to women with a diagnosis of PMS according to established diagnostic criteria DSM-III-R. 170 patients were enrolled and evaluated at the end of the study, 86 on active and 84 on placebo. The main efficacy variable was the change from baseline to endpoint in the Patients' Self-Assessment (PSA) of 6 main PMS symptoms. Secondary efficacy variable was changes in Clinical Global Impression (CGI) with the items severity of condition, global improvement and risk/benefit. Response to treatment was defined as >50% reduction in symptoms at endpoint.

Patients who received Ze440 had a significant improvement in the combined symptoms of PMS, relative to those on placebo. The PSA scores were corroborated by the physicians' own evaluation of all 3 items of the CGI scale. 5 of the 6 PSA-items showed significant superiority for Ze440 (irritability, mood alteration, anger, headache and breast fullness). The responder rate was also favourable for the treated group relative to placebo (52% vs 24%, respectively).

Meanwhile, the product has been successfully introduced under the brand names Prefemin® and Premens® in the Swiss market and the response is favourable in prescription as well as in self medication.

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