

PROTECTIVE EFFECT OF AN ORAL NATURAL PHYTONUTRIENT IN RECURRENT VULVOVAGINAL CANDIDIASIS: A 12-MONTH STUDY

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The aim of the present study is to assess the clinical efficacy of a phytochemical with antimicrobial properties (K-712, with the following 100 mg composition: 10 mg of oleoresin from *Pseudowintera colorata* at 30% concentration in Polygodial together with trace amounts of *Olea europea*) in recurrent vulvo-vaginal candidiasis (RVVC) as compared to an azole drug during a 12-month period: 6 months of treatment followed by 6 months of observation. This prospective randomized study involved 82 women (19-61 years) with complaints of abnormal vaginal discharge and with a history of at least four proven episodes of RVVC in the previous 12 months. Patients were divided into two groups of treatment of 41 patients each and were given: A) Itraconazole 200 mg orally daily for 4 days, then 200 mg once weekly for 6 months or B) 1 tablet twice a day of a K-712 for 4 weeks and then for the first 2 weeks of each month for a total of 6 months. Both groups were then followed-up for further 6 months. Each treatment schedule was well tolerated with only 4 patients in the azole group complaining of transient mild symptoms (nausea, abdominal discomfort, unpleasant taste). Itraconazole reached an earlier symptomatic relief during the first two weeks of observation as compared with K-712 ($p < 0.05$) but both treatments enabled a comparable benefit during the entire treatment study period, afterwards with comparable symptom/sign score (itraconazole vs K-712: 9 vs 11). At 6-month observation, mycological cure was reached by 83% in the itraconazole group and in 78% of the K-712-treated patients. During the further 6-month observation period without treatment, the itraconazole group showed significantly more relapses (65.7 vs 34.2 in K-712, $p < 0.05$) and at the end of the whole 12-month study period the mycological cure was significantly higher in the K-712-treated patients (65.8% vs 34.3%, $p < 0.05$). There was a non-significant trend increase of less drug-susceptible species in the itraconazole group. From these preliminary data it would appear that a natural antifungal phytochemical proves to be as good as itraconazole in the maintenance treatment of RVVC. Moreover, this approach seems to maintain a higher mycological success rate afterwards by reducing the number of relapses and probably of the growth of azole-resistant species.

Key words: recurrent vulvovaginal candidiasis, itraconazole, antifungal phytonutrient

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Candida species have emerged as clinically important pathogens associated with opportunistic infections causing common ailments such as thrush and vaginitis, as well as chronic conditions in immunocompromised patients (1), and this explains the widespread use of azole drugs, especially fluconazole. However, repeated fluconazole therapy for fungal infections in patients, such as women with *C. albicans* infections, has been associated with an increase in azole resistance, and the survey shows that about 30% clinical *C. albicans* isolates from women with vaginitis are resistant to fluconazole (2-3). In particular, 3.6-7.2% of vaginal isolates of *Candida* spp. from women with *Candida* vaginitis are resistant to fluconazole. Moreover, the repeated use of azole drugs can lead to colonization with less susceptible species. Indeed, the prevalence of non-*C. albicans* strains is increasing over time and these strains are more likely to be found in women reporting recurrent vulvovaginal candidiasis.

In clinical practice, recurrent vulvovaginitis (RVVC) is defined as four or more episodes of VVC in one year, with at least one of these episodes well documented with culture, and most cultures being positive for *C. albicans* (Table I). Seventy percent of women with RVVC who are treated with a conventional antifungal treatment can expect to have another episode within 6 months (4). For the most part, self-treatment of repeated episodes allows rapid initiation of antimycotic therapy but it does little to prevent the next attack. Therefore, the most effective approach to treatment, particularly with *C. albicans* infections, seems to rely on antifungal maintenance therapeutic strategy. Treatment options, which have been studied and shown to be effective, include ketoconazole (100 mg orally daily), clotrimazole (500 mg suppositories weekly) (5), itraconazole and fluconazole (200 mg or 150 mg orally once weekly, respectively) (6). Because of liver toxicity associated with use of ketoconazole, the latter two agents, especially fluconazole, are now preferred as maintenance regimens although this drug is more expensive.

With the view to minimizing further interference with the existing biological ecosystem secondary to the use of chemically synthesized antifungal molecules (7-9), a number of different approaches, such as the use of phytochemicals, have been

under investigation (10-11). In the present study we used a natural phytotherapeutic agent based on anethole/polygodial, which we have previously shown to significantly inhibit *Candida* adhesiveness to duodenal mucosa as well as limiting its luminal translocation while being devoid of toxicity on an experimental level (12-13).

During the past decade, fluconazole has been mainly used in high-risk populations, such as neutropenic patients and, together with its unquestionable benefits against systemic candidiasis, this has also led to a selection-driven shift from highly susceptible to less susceptible *Candida* species. Polygodial, a component of *Pseudowintera colorata*, has long been used as a phytotherapeutic agent for its antibacterial and antifungal properties. In recent years an anethole/polygodial compound has been clinically used since it has been found that anethole, a natural compound isolated from *Pimpinella anisum*, enables an over 30-fold increase in the antifungal activity of polygodial against *C. albicans* (14). We have conducted some studies proving the efficacy of such phytocompound in several experimental set ups (15-16). Thus, the aim of the present study is to assess the clinical efficacy of this compound in RVVC as compared to conventional drug approach with an azole drug throughout a 12-month period.

MATERIALS AND METHODS

This prospective randomized study involved 82 women (19-61 years), out of the initially 109 found eligible, with overall general health, who presented to the gynaecology outpatient clinic with complaints of abnormal vaginal discharge and with a history of at least four proven episodes of VVC in the prior 12 months.

The presence of vaginal discharge was confirmed by a speculum examination. Women who were pregnant, <6 weeks post-abortion or post-partum, diabetic, immunocompromised, on chronic drug therapy like steroids or antibiotics, pelvic inflammatory disease and diagnosed or suspected genital malignancy were excluded.

The random numbers were computer generated and patients were allocated into two groups of treatment of 41 patients each. Patients were given A) Itraconazole 200 mg orally daily (administered as 100 mg twice daily with meals) for 4 days, then 200 mg once weekly

for 6 months, or B) 1 tablet twice a day of a polygodyal/anethole phytocompound (K-712, supplied by Canova Foundation, Lesmo, Italy); the 100 mg composition is: 10 mg of oleoresin from *Pseudowintera colorata* at 30% concentration in polygodial together with trace amounts of *Olea europea* for 4 weeks and then for the first 2 weeks each month for a total of 6 months. Both groups were then followed-up for further 6 months. Typical symptoms and signs of genital infection by *Candida* yeasts (itching, burning, dyspareunia, curd-like vaginal discharge, urinary symptoms, reddened genital mucosa with erythema, oedema, fissures and whitish patches) were each scored by means of the intensity (absent = 0; mild = 1; moderate = 2 and severe = 3).

The primary outcome was the comparison between K-712 phytocompound and a synthetic antifungal drug in the symptomatic relief and mycological cure in women with laboratory evidence of infection in treatment of individual vaginal infections during treatment and the follow-up period. The secondary outcome was the study of rate of mycological cure at the end of the whole 12-month study, the rate of recurrence during the 6-month observation period and the possible microbiological shift of less drug-susceptible *Candida* strains. Tolerability was also assessed.

Biological sampling and processing

Using a sterile Cusco's speculum and cotton swabs, vaginal discharge was collected from the posterior fornix for culture of yeasts and anaerobes and samples were transferred to the laboratory within 6 h where they were dyed with Gram technique and examined microscopically to observe yeasts and pseudohyphae characteristic of *Candida* infection. Vaginal pH test: a vaginal swab from the posterior fornix was touched onto pH testing paper strips with a range 4.0–10.0 (Merck, Germany). Wet-mount smear: a drop of vaginal discharge was placed on two glass slides, diluted with a drop of 0.9% saline, covered with a cover slip and examined under high power. A vaginal identification of pathogens wet preparation with 0.2% crystal violet stain was carried out on one slide to identify clue cells. Culture: the vaginal swabs were inoculated onto blood agar and human blood agar for growth of *Gardnerella vaginalis* and on Sabouraud's dextrose agar (SDA) for growth of yeasts. The organisms were identified according to colony morphology and standard methods. Efficacy of the treatment was assessed during follow-up based on symptomatic relief, pelvic examination and laboratory investigations in the form of normal wet smear, pH of 4.0 and absence of any growth on cultures. Absence of abnormal vaginal discharge in women reporting relief in symptoms was confirmed by a speculum examination.

The identification of *Candida* species included: 1) formation of germinative tube: a sample of the culture in SDS medium was inoculated into 2.5 mL of human serum; this was resuspended and incubated for 3 h at 37°C to observe the pleiomorphism via microscopy; 2) production of chlamydoconidia, hyphae, and pseudohyphae: a sample of the same SDS medium was spread on Chlamydiospore Agar medium and was covered with a sterile slide, the culture was incubated at 27 and 37°C for 48 h; hyphae and chlamydoconidia were observed under a microscope; 3) coloration in ChromAgar: a cellular suspension was adjusted to tube 3 of the McFarland Nefelometer, 5 µL were inoculated into ChromAgar *Candida* medium (Becton-Dickinson) and incubated for 78 h at 37°C; *Candida* species were identified based on characteristic color; 4) the biochemical identification of the species was made with the API-20C-test.

Efficacy of the treatment was assessed during follow-up based on symptomatic relief, pelvic examination and laboratory investigations in the form of normal wet smear, pH of 4.0 and absence of any growth on cultures. Absence of abnormal vaginal discharge in women reporting relief in symptoms was confirmed by a speculum examination.

Statistical analysis

Only patients adhering to the protocol were analysed for efficacy; patients lost to follow-up or non-compliant with the treatment regimen were excluded from analysis; patients lost to follow-up or who had recurrences due to non-compliance were assessed as failures. For continuous variables the groups were compared using Student's *t* test. For categorical variables, the groups were compared by *Chi*-square analysis. Ninety-five percent confidence intervals were calculated for all results expressed as rates.

RESULTS

In our study all vaginal mycotic infections were confirmed to be caused by yeasts of the genus *Candida*. No major side effects or other clinical conditions requiring interruption of treatment were seen in either group. Each treatment schedule was well tolerated, with only 4 patients in the azole group complaining of transient mild symptoms (nausea, abdominal discomfort, unpleasant taste). Four patients in each group were excluded for the following reasons: 2 lost to follow-up, 3 for major violation of protocol (1 use of antibiotics, 1 use of topical antifungal cream and 1 use of antiseptic vaginal irrigation) and three for low compliance (one during the 6-month treatment period and 2 during the

Table I. Classification of vulvovaginal candidiasis.

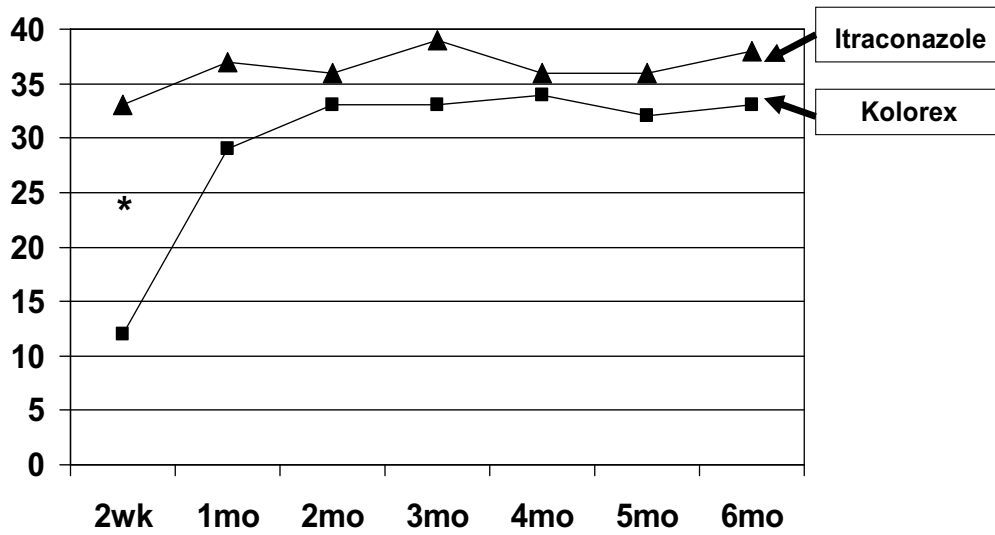
Uncomplicated
Sporadic or infrequent and
Mild-to-moderate symptoms or findings and
Suspected <i>C. albicans</i> infection and
Normal, non-pregnant woman
Complicated
Recurrent (≥ 4 per year) episodes or
Severe symptoms or findings or
Suspected or proven non- <i>albicans</i> <i>Candida</i> infection or
Abnormal host (diabetes, severe medical illness, immunosuppression, other vulvovaginal conditions or pregnancy)

Data from Centers for Disease Control and Prevention, Workowski KA, Bernan SM. Sexually transmitted diseases treatment guidelines, 2006. *MMWR Recomm Rep* 2006 4;55(RR-11):54.

further 6-month follow-up). Altogether, 38 patients in each group were examined and considered for final calculation of the data. From the overall analysis it appeared that while itraconazole reached an earlier symptomatic relief during the first two weeks of observation compared to K-712 (86.8 vs 31.5, $p < 0.05$), both treatments enabled a comparable benefit during the whole treatment and study period (Fig. 1). In particular, when analysing the intensity of symptoms in patients who were still symptomatic during treatment, there was no significant difference between the two groups (symptom/sign score in itraconazole vs K-712: 9 vs 11, data not shown).

As for mycological eradication, at the first monthly check-up the confirmed mycological cure was reached by 97.3% of the patients treated with itraconazole with a significantly better performance compared to the 63.1% observed in K-712 ($p < 0.01$

(Fig. 2). However, starting from the 2-month observation during the first 6-month treatment phase, both treatments showed a comparable benefit. Moreover, during the observation period, mycological cure was observed in a significantly higher number of patients as compared to itraconazole-treated ones with a final 12-month percentage of 65.8% vs 34.3% ($p < 0.05$, Fig. 2). During the overall 12-month study, and mainly during the 6-month observation phase, significantly more episodes of clinical RVVC were reported in patients who had previously been treated with itraconazole than with K-712 ($p < 0.05$, Fig. 3). A higher growth of less drug-susceptible species was observed in the itraconazole group but this trend did not reach a statistical significance (67.6% vs 50%). There was no apparent relationship between the ages of patients and the response to each of the therapeutic regimens or the occurrence of recurrence



* $p < 0.05$ vs itraconazole

Fig. 1. Number of patients reporting symptomatic relief during 6-month treatment period.

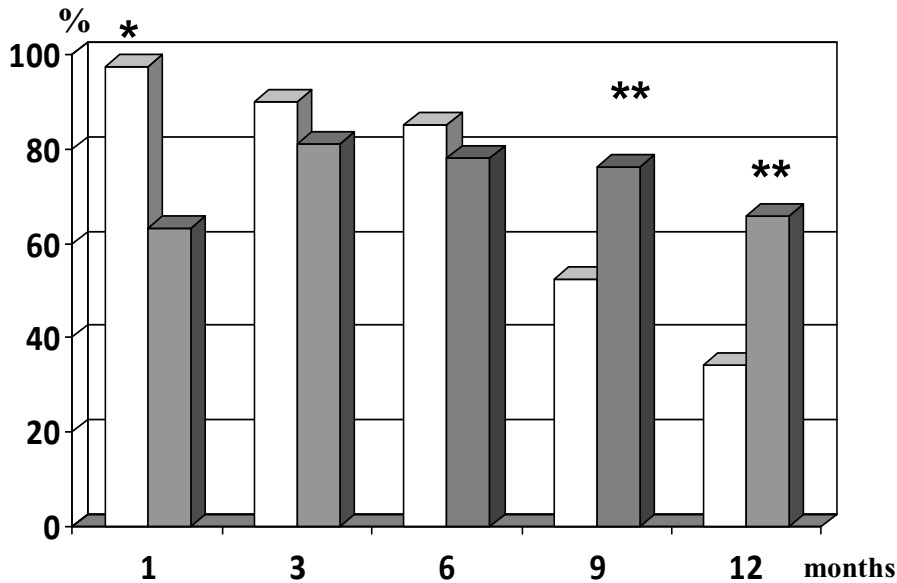


Fig. 2. Number of mycologically “cured” patients during 12-month treatment and follow-up period. White bars: Itraconazole group; Grey bars: K-712-treated group. * Itraconazole vs K-712, $p < 0.05$; ** K-712 vs Itraconazole, $p < 0.05$

(data not shown).

DISCUSSION

Vulvovaginal candidiasis (VVC) is a common

problem, causing significant morbidity and affecting women’s wellbeing. Candida is a saprophytic opportunistic microorganism and condition resulting in a decrease in vaginal pH or alteration of the local defense mechanisms, favouring the appearance

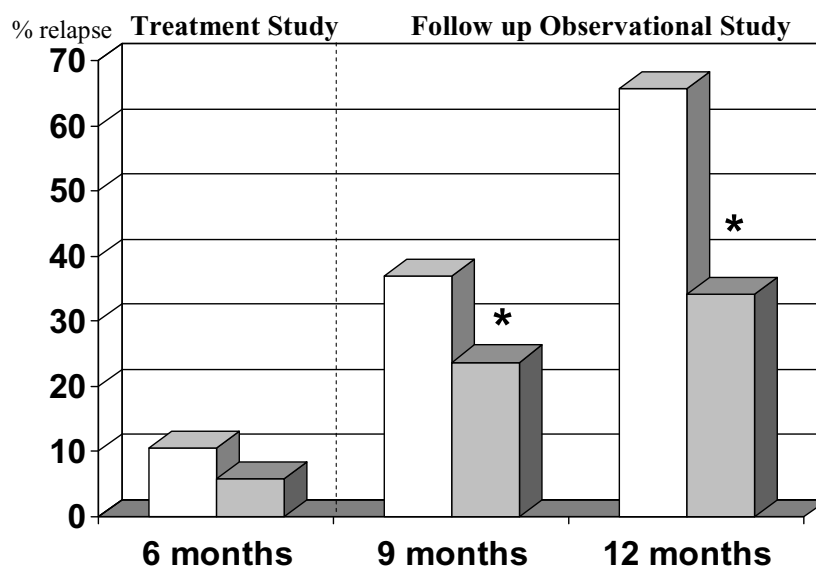


Fig. 3. Relapse rate of RVVC in patients treated with itraconazole or k-712 during 12-month study period. White bars: Itraconazole group; Grey bars: K-712-treated group. * $p < 0.05$ vs itraconazole-treated group

of *Candida* vaginitis. For clinicians involved in women's health care, vaginitis is a commonly reported complaint and one of the most frequent causes for patient visits to obstetrician-gynecologists (17). Of the many reasons for vaginal infections, bacterial vaginosis and vulvovaginal candidiasis (VVC) are regarded to be the two most common clinical situations, accounting for an estimated 22% to 50% and 17% to 39% of symptomatic women, respectively (18).

It has to be pointed out that women who harbor *Candida* organisms in their vaginas have VVC, with a spectrum of manifestations ranging from a virtually asymptomatic colonization to severe acute symptomatic infection. Indeed, yeast colonization occurs quite frequently, with up to 30% of healthy asymptomatic women showing a positive culture for yeast at any random observation and up to 70% if followed longitudinally over a 1-year period. In our study we made sure to recruit patients with proven and recorded history of RVVC and with overt symptoms and biological proof of active VVC.

Although several chemically synthesized antifungal molecules have proven their efficacy in clinical practice, nonetheless they also have some drawbacks such as toxic effects and opportunistic

bacterial overgrowth (19). As a matter of fact, the azole drugs, especially fluconazole, are widely used to treat *C. albicans* infections. Understandably, repeated fluconazole therapy for fungal infections in patients, such as vaginitis in women with *C. albicans* infections, has been associated with an increase in azole resistance and a recent survey showed that 33.5% of clinical *C. albicans* isolates from women with vaginitis were resistant to fluconazole (20). Moreover, several other *Candida* species, such as *C. krusei* and *C. tropicalis* are inherently resistant to fluconazole (21). Therefore, it is very important to find antifungal drugs with a novel chemical structure. For this reason, natural products active against *Candida* spp. have increased significantly in the last 10 years, and approximately 258 plant species, from 94 families have been examined (22).

From these data it would appear that a natural antifungal phytochemical proves to be as good as itraconazole in the maintenance treatment of RVVC although azole drugs are likely to be more effective in very early symptomatic and mycological improvement, as also shown in our study.

However, when looking at a 1-year follow up, it appears that the itraconazole-treated group showed significantly more relapses than the group given

Table II. *Candida* isolated from patients with RVVC: frequency and species.

species	Itraconazole (at entry: 38)	Itraconazole (all relapses:34)	K-712 (at entry: 38)	K-712 (all relapses:14)
<i>C. albicans</i>	20 (52.6 %)	11 (32.3 %)	21 (55.2%)	7 (50.0%)
<i>C. tropicalis</i>	1 (2.6%)	1 (2.9%)	3 (7.8%)	-
<i>C. glabrata</i>	12 (31.5%)	16 (47.0%)	9 (23.6%)	4 (28.5%)
<i>C. guilhermondii</i>	-	1 (2.9%)	-	-
<i>C. parapsilosis</i>	1 (2.6%)	2 (5.8%)	3 (7.8%)	2 (14.2%)
<i>C. krusei</i>	4 (10.5%)	6 (17.6%)	2 (5.2%)	1 (7.1%)
Other candida species	-	1 (2.9%)	-	-
Overall non- albicans strains	47.3%	67.6%	44.7%	50.0%

K-712 ($p < 0.05$). Moreover, when checking the growth of less drug-susceptible species there was a difference between the two groups, in favour of the K-712-treated group although the small number did not allow a statistical analysis. This is relevant when considering that hepatitis has been associated with ketoconazole, and the risk of liver injury has been estimated at one in 15,000 people, especially for women over 50 years of age. A further issue to address is whether between the two treatment options there is any significant difference for non-albicans *Candida* infection, which is a recent concern in the field (23). Although *C. albicans* is known to be the dominant species of pathogenic yeasts isolated from women attending gynaecology clinics, as expected in such recurrent cases, the frequency of non-albicans species at entry was elevated, with an

average of 46% (47.3% and 44.7% in itraconazole and K-712 groups, respectively) (Table II). Among the non-albicans, *C. glabrata* was the most prevalent species.

When pooling together the species isolated during the relapse infection episodes along the 1-year follow-up, the non-albicans species in the itraconazole-treated patients were the majority (67.6%), in which the etiologic agent was *C. glabrata* in 47%, *C. albicans* in 32.3% and *C. krusei* in 17.6% of cases. This seems to confirm the suggestion that repeated exposure to antifungal agents may eventually cause a shift in the vaginal mycoflora from the more drug-susceptible *C. albicans* to the less drug-susceptible species. Indeed, several reports suggest that these non-albicans species are generally more resistant to imidazole and polyene therapy than *C. albicans* and

that the therapy itself may create the non-albicans selection (24).

Overall, our study suggest that in vulvovaginal candidiasis and especially in those recurrent cases, a phytotherapeutic option is a valid option while a larger population and longer follow-up may ascertain whether a weekly phytotherapeutic-based maintenance treatment may represent an effective tentative preventive measure.

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