Proanthocyanidin polymeric tannins from Stryphnodendron adstringens are effective against Candida spp. isolates and for vaginal candidiasis treatment

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A B S T R A C T

Ethnopharmacological relevance: The stem bark of Stryphnodendron adstringens (Mart.) Coville is popularly used as anti-inflammatory, astringent and in the treatment of wounds and vaginal infections. Several pharmacological activities have been scientifically proven by in vitro and in vivo experimental assays for antibacterial, antiviral, antiprotozoan, anti-inflammatory and antioxidant.

Aim of the study: We investigated whether proanthocyanidin polymeric tannins from the Stryphnodendron adstringens stem bark with antifungal activity against Candida albicans in vitro are also active against planktonic and biofilm cells of Candida non-albicans (CNA), including fluconazole-resistant isolates and are capable of controlling Candida vaginitis in vivo.

Materials and methods: A total of 46 clinical isolates and 5 reference Candida spp. strains were used in this study. The antifungal effects of tannins (F2 and sub-fraction F2.4) from S. adstringens stem bark were evaluated using a broth microdilution assay (for planktonic yeasts and biofilm dispersion cells) or by XTT assay (for biofilm sessile cells). For in vivo antifungal activity analysis, mice with vaginal infection by C. albicans or C. glabrata were treated with a topical gel containing F2 (alone or combined with oral fluconazole), and the vaginal histopathology and fungal burden (by CFU counts from vaginal homogenates) were analyzed.

Results: F2 and F2.4 inhibited the proliferation of planktonic cells of Candida spp., especially that of fluconazole-resistant isolates. F2 and F2.4 also inhibited the proliferation of Candida biofilm dispersion cells. Moreover, a gel containing F2 efficiently controlled vaginal infection by C. albicans and C. glabrata in mice, with no noticeable toxicity to vaginal tissue.

Conclusions: Our data show that proanthocyanidin polymeric tannins obtained from S. adstringens have antifungal activity in vitro against C. albicans and CNA (including fluconazole-resistant isolates) and presented efficacy in the control of candidiasis in murine model. Therefore, these tannins have potential use in the treatment of vaginal candidiasis, representing interesting alternatives to current antifungals.

1. Introduction

Candidiasis is an infection caused by Candida spp. that may occur in the skin, eyes, oral cavity, oesophagus, gastrointestinal tract, vagina and vascular system. Vulvovaginal candidiasis (VVC) is the second most common vaginal infection, with 50–75% of women suffering from VVC at least once in their lifetime and 5–8% developing a chronic and severe form known as recurrent vulvovaginal candidiasis (RVVC) (Cassone, 2015; Sobel, 2016). Approximately 90% of Candida isolates from vaginal fluid are identified as Candida albicans, while the minority of cases are caused by Candida non-albicans (CNA); however, the number of fungal vaginitis cases caused by CNA has increased over the years...
VVC caused by CNA species are clinically indistinguishable from those caused by *C. albicans*. On the other hand, RVVC may be caused by isolates that are either less sensitive or resistant to antifungals, from species such as *Candida glabrata*, which affect 10–30% of women (Pappas et al., 2016; Sobel, 2016). In addition to resistance, *Candida* biofilm formation on the vaginal mucosa could also facilitate chronic disease establishment by persistent and antifungal resistant cells present in the biofilms (Muzzy and Schwebeke, 2015; Sobel, 2015). Although few strains resistant to antifungal agents have been isolated from vaginal fluid, VVC and RVVC remain a common problem for healthy and immunocompetent women, affecting their quality of life (Sobel, 2016).

The available antifungal therapy used to treat VVC and RVVC is based on the topical and/or oral administration of polyenes and azoles; however, these drugs have a narrow spectrum of action and considerable toxicity (Pappas et al., 2016). Therefore natural products have been studied as sources of new agents and leads which may contribute to improve the activity and decrease the toxicity of antifungal treatment (Crag and Newman, 2013; Harvey et al., 2015). Some natural compounds or derivatives, such as polyenes and echinocandins, are currently used to treat invasive fungal infections (Dutcher, 1968; Emri et al., 2013), and have shown efficacy against vaginal candidiasis species *in vitro* (Boikov et al., 2017) and *in vivo* (Stevens et al., 2002).

*Stryphnodendron adstringens* (Mart.) Coville, known as “barbatimão”, is a plant commonly found in the Brazilian savannah and the ethnomedicinal use of *S. adstringens* barks is associated with the treatment of inflammatory and infectious diseases in the decoction, infusion and tincture preparations (Brasil, 2014, 2010; Santos et al., 1987). These preparations are generally administered by topical way for treatment of skin wounds, skin and urinary infections, and urethral and vaginal infections. Importantly, these extracts present a relevant effect in the reduction of vaginal inflammation and leucorrhea, two important symptoms related to vaginal infections (Brasil, 2014). In addition, anti-inflammatory and antimicrobial activities of these extracts have been experimentally confirmed *in vitro* and *in vivo* (Brasil, 2014), including antifungal effects against molds (*Fusarium oxysporum, Cladosporium sphaerospermum, Trichophyton rubrum,* and *Pythium insidiosum*) (Lanchoti Fiori et al., 2013; Melo-Silva et al., 2009; Trolezi et al., 2017) and yeasts (*Candida* spp. and *Cryptococcus neoformans*) (Ishida et al., 2009, 2006; Luiz et al., 2015; Morey et al., 2016).

Previously, we showed that proanthocyanidin polymeric tannins present in the aqueous fraction F2 and semi-purified subfraction F2.4 from the stem bark of *S. adstringens* (Fig. 1) have antifungal activity *in vitro* against planktonic cells of *C. albicans* (Ishida et al., 2006) and impaired *C. albicans* biofilm formation (Luiz et al., 2015). However, these tannins had not been tested against CNA and resistant isolates, and had not been tested *in vivo*, in the treatment of vaginal candidiasis. Here, we tested the antifungal activity *in vitro* of F2 and F2.4 from the *S. adstringens* stem bark against planktonic yeasts and (dispersion and sessile) biofilm cells of *C. albicans* and CNA clinical isolates (including fluconazole-resistant isolates). Furthermore, a vaginal gel formulation containing fraction F2 was tested *in vivo*, in the treatment of vaginal candidiasis by *C. albicans* and *C. glabrata*.

### 2. Materials and methods

#### 2.1. Candida strains

Clinical isolates (obtained from the Adolfo Lutz Institute - IAL, São Paulo, SP, Brazil) from the following *Candida* spp. were used in this study: *Candida albicans* (n = 7), *C. tropicalis* (n = 9), *C. parapsilosis* (n = 8), *C. glabrata* (n = 10), *C. krusei* (n = 9), and *C. guilliermondii* (n = 3) (“IAL” isolates; Table S1). The following reference strains were also included: *C. albicans* (SC5314 and ATCC 10231), *C. parapsilosis* (ATCC 22019), *C. tropicalis* (ATCC 200956), *C. glabrata* (ATCC 2001), and *C. krusei* (ATCC 6258). All strains were maintained at ~ 80 °C, recovered in Sabouraud dextrose agar plates (Becton Dickinson and Company, Sparks, USA) and subcultured twice in the same medium at 35 °C for 48 h, before each assay.

#### 2.2. Tannin extraction and vaginal gel formulation

Stem bark samples from *S. adstringens* were collected in the city of São Jerônimo da Serra (PR, Brazil), and a voucher specimen (HEUM #14321) was deposited at the Herbarium of the State University of Maringá (HEUM, Maringá, PR, Brazil). Fraction F2 and subfraction F2.4 were prepared as described previously (Ishida et al., 2006) and kept lyophilized at ~ 20 °C, in 1-mg aliquots. The identities of the proanthocyanidin polymeric tannins in the semi-purified subfraction F2.4 (Fig. 1) were confirmed using mass spectrometry and compared with the results reported by Ishida et al. (Ishida et al., 2006).

Vaginal gel formulations containing fraction F2 (2.5% or 5%, w/w) were prepared by dispersing Carbopol-940® polymer (1.0%) in sterile distilled water. Methylparaben (0.2%) and sodium carbonate were added as a preservative and a neutralizer, respectively. Then, F2 fraction (2.5% or 5%, w/w) was incorporated (Kaplum et al., 2012).

#### 2.3. Standard antifungal agents

Amphotericin B (AMB), miconazole (MCZ), and fluconazole (FCZ) (all from Sigma Chemical Co., USA) were used as reference antifungals. For *in vitro* assays, AMB and MCZ stock solutions of 1600 mg/L were prepared in dimethyl sulfoxide, and a 2560 mg/L stock solution of FCZ was prepared in sterile distilled water (stock solutions were stored at ~ 20 °C). For vaginal applications, MCZ (2%, w/w) was incorporated into a vaginal cream (10% Wax self-nionic emulsifier, 2% mineral oil, 5% propylene glycol, and 84% distilled water, pH 4.5). FCZ to be used in oral administration was diluted to 6000 mg/L in sterile distilled water.

#### 2.4. Antifungal susceptibility assay for planktonic cells

The antifungal susceptibility of planktonic cells of *Candida* spp. was assessed by the broth microdilution assay as described in the documents M27-A3 (Clinical and Laboratory Standards Institute, 2008) and M27-S4 (Clinical and Laboratory Standards Institute, 2012). The concentrations tested were 0.48–250 mg/L for fraction F2 and subfraction F2.4, 0.12–64 mg/L for FCZ, and 0.03–16 mg/L for MCZ and AMB. The minimal inhibitory concentration of antifungals on planktonic cells (MIC) value was defined as the lowest concentration that inhibited 50% (PMIC50, for F2, F2.4, FCZ, and MCZ) and 90% (PMIC90, for AMB) of fungal growth determined by spectrophotometric reading at 492 nm (Epoch 2, Biotek Instruments, USA). After treatments, a 10 µL aliquot of treated fungal samples was plated on drug-free Sabouraud dextrose agar plates and incubated at 35 °C for 48 h, to determine the minimal fungicidal concentration (MFC), defined as the lowest concentration of antifungal that killed 99.9% of fungal cells. The effect was considered fungicidal when MFC ≤ 4x PMIC; otherwise, the effect was considered fungistatic. All experiments were performed as three independent replicates.

#### 2.5. Antifungal combination assay

Combinations of fraction F2 or subfraction F2.4 with AMB, FCZ or MCZ were tested against planktonic cells of *Candida* spp. reference strains using the Checkerboard broth microdilution method, in 96-well plates (Pillai et al., 2005). Fractional Inhibitory Concentration index (FIC) values were calculated as described previously. The treatment effect was considered synergistic when FIC ≤ 0.5, indifferent when 0.5 < FIC < 4.0, and antagonistic when FIC ≥ 4.0 (Pillai et al., 2005). All experiments were performed as three independent replicates.
2.6. Antifungal treatment on Candida biofilms

Antifungal assays on biofilms were performed with two isolates of each Candida species tested here (an FCZ-susceptible and an FCZ-resistant isolate, whenever possible). The antibiofilm activity of antifungals was evaluated in two phases of biofilm development, during biofilm formation and on pre-formed biofilms. All experiments were performed in three independent replicates, and in triplicates. For biofilm formation, 10⁷ colony forming units (CFU)/mL suspensions of Candida yeasts in RPMI 1640 medium with 0.16 M MOPS (pH 7.0) (both from Sigma Chemical Co., USA) were prepared, and 100 µL aliquots each suspension were dispensed per well, in 96-well flat microplates. Plates were incubated for 1.5 h at 35 °C and shaking (150 rpm), for fungal adhesion phase. Then, the supernatant was removed, wells were washed twice with PBS, and 100 µL of RPMI 1640 medium were added to each well, to allow biofilm formation by incubation at 35 °C with shaking (150 rpm), for 24 h.

To evaluate antifungal activity on biofilm formation, 100 µL of RPMI 1640 medium containing different concentrations of F2, F2.4, AMB, FLZ, or MCZ were added to each well after the adhesion phase, and the plates were incubated for 24 h at 35 °C, with shaking (150 rpm).

To evaluate the effect of antifungals on sessile cells of pre-formed biofilms, supernatants (containing dispersion cells) were removed from
each well after 24 h of incubation, and the sessile cells were treated with antifungals for 24 h at 35 °C, with shaking (150 rpm).

After treatments, the metabolic activity of biofilm cells was quantified using the 2,3-bis(2-methoxy-4-nitro-5-sulfo-phenyl)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide (XTT) reduction assay (Luiz et al., 2015), and the results were used to determine minimal inhibitory concentrations on sessile cells from biofilms (BMIC<sub>50</sub> and BMIC<sub>90</sub>, corresponding to concentrations that inhibit 50% and 90%, respectively, of the metabolic activity of biofilm cells).

The supernatants removed from pre-formed biofilms were used to evaluate the antifungal susceptibility of dispersion cells by the determination of dispersion cell MIC values (DMIC<sub>50</sub> and DMIC<sub>90</sub>), by the same method used to determine the antifungal susceptibility of planktonic cells (see “Antifungal susceptibility assay on planktonic cells”).

### 2.7. Antifungal activity against vaginal candidiasis in vivo

Female BALB/c mice (6–8 weeks old) from the Biomedical Sciences Institute (ICB, University of São Paulo, SP, Brazil), were maintained in pathogen-free conditions, with food and water “ad libitum”, and in accordance with National Institute of Health Animal Care Guidelines (MCTI-CONCEA, 2013). All procedures were previously approved by the local Ethics Committee for Animal Use (CEUA, ICB, USP, Protocol no. 039-3-5).

The pseudo-osiruous phase in mice was induced by the subcutaneous administration of 0.5 mg of 17-beta-estradiol valerate (in sesame oil), 3 days before vaginal infection initiation (Hamad et al., 2004). Yeasts of C. albicans ATCC 10231 or C. glabrata ATCC 2011 were cultivated in Sabouraud dextrose broth, harvested and diluted to 3 × 10<sup>8</sup> CFU/mL in C. albicans ATCC 10231 or days before vaginal infection initiation (Hamad et al., 2004). Yeasts of administration of 0.5 mg of 17-beta-estradiol valerate (in sesame oil), 3 no. 039-3-5).

(MCTI-CONCEA, 2013). All procedures were previously approved by accordance with National Institute of Health Animal Care Guidelines pathogen-free conditions, with food and water “ad libitum”, and in

Institute (ICB, University of São Paulo, SP, Brazil), were maintained in

In order to determine the fungal burden, mice were euthanized and the vaginas were aseptically dissected, weighed, and homogenized in sterile PBS. Vaginal infections were initiated by intravaginal inocula-

Table 2

<table>
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<th>Species</th>
<th>Isolate</th>
<th>F2</th>
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<th>FCZ</th>
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<td>C. glabrata</td>
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<td></td>
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<td>0.48</td>
<td>1.95</td>
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<tr>
<td></td>
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<td>7.81</td>
<td>0.81</td>
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<tr>
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<td>15.63</td>
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<td>15.63</td>
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<td>1.95</td>
<td>0.98</td>
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<tr>
<td></td>
<td>IAL-01</td>
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<td>31.25</td>
<td>250</td>
<td>31.25</td>
<td>0.5</td>
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</tbody>
</table>

* susceptible; R resistant; see susceptible dose-dependent.

3. Results and discussion

3.1. Proanthocyanidin polymeric tannins from Stryphnodendron adstringens stem bark inhibit the proliferation of planktonic cells from Candida non-albicans clinical isolates, including fluconazole resistant strains

The emergence of Candida species resistant or less susceptible to the antifungals currently used to treat VVC/RVVC, as well as the narrow spectrum of action and the high toxicity of these drugs, have increased the need for new alternatives – including natural compounds - to treat fungal vaginitis (Pappas et al., 2016; Sobel, 2016). Antifungal properties of fraction F2 and subfraction F2.4 from S. adstringens stem bark, composed of flavan-3-ol units arranged as 6 U with one or more gallic acid units and interconnected by linkages 4→8 and 4→6 (Fig. 1), had been demonstrated previously by our research group (Ishida et al., 2006; Luiz et al., 2015), but these natural compounds had not been tested against CNA species or in vivo, in the treatment of vaginal infections.

As an initial step to examine whether fraction F2 and subfraction F2.4 have antifungal activity against CNA, we determined PMIC and MFC values for the treatment of planktonic yeasts from a total of 39 CNA clinical isolates in the Experimental model of Disseminated Cell Infammation System (MCIS) and 4 reference strains, and compared with the treatment of C. albicans clinical isolates (Table 1 and S1). The susceptibility of these isolates to standard antifungals had been tested previously (Muñoz et al., 2017). AMB and MCZ had the best antifungal activity against all CNA isolates tested (PMIC values ≤ 2 mg/L for AMB and ≤ 8 mg/L for MCZ), except for the four C. albicans strains considered resistant to AMB (ATCC 200956, ATCC 6258, and IAL-21) and for the three CNA isolates less susceptible to MCZ (ATCC 200956, ATCC 2001, SCS314, IAL-44) (Muñoz et al., 2017) (Table 1 and Table S1). In contrast, several CNA and C. albicans isolates were resistant to FCZ (Muñoz et al., 2017) (Table 1 and Table S1).

Our data confirm that C. albicans strains are susceptible to F2 and
polymeric tannins are more effective for yeasts (Candida and Cryptococcus) than filamentous fungi, wherein the inhibitory effect was only observed in higher concentrations (6000 to 10000 mg/L) of fractions from S. adstringens stem bark (Lanchoti Fiori et al., 2013; Melo-Silva et al., 2009; Trolezi et al., 2017).

Similarly to that observed for FCZ and MCZ (Muñoz et al., 2017), both F2 and F2.4 had fungistatic activity against C. albicans and CNA clinical isolates (MFC > 250 mg/L), while the effect of AMB was fungicidal (Muñoz et al., 2017) (Table 1 and Table S1). The combination of F2.4 (0.49 mg/L) with FCZ (1 mg/L) had a synergistic effect on C. glabrata ATTC 2001 (FIC Index = 0.5). However, all other antifungal drug combinations tested against Candida spp. were considered indifferent (0.5 < FICI ≤4).

3.2. Proanthocyanidin polymeric tannins have antifungal activity against dispersion cells from Candida spp. biofilms

The formation of biofilms on mucosal surface may occur in vaginal candidiasis (Muzny and Schwebeke, 2015), and biofilm formation is important for pathogenesis (Nobile and Johnson, 2015). Moreover, biofilm formation is associated with chronic infection, due to the lower antifungal susceptibility of biofilms cells, and to the ease of disease dissemination by dispersion cells that become detached from established biofilms (Nobile and Johnson, 2015).

We assayed the anti-biofilm activity of F2 and F2.4 (compared with that of standard antifungals) against CNA and C. albicans biofilms in two different phases of development: during biofilm formation and on pre-formed biofilms (dispersion and sessile cells). For biofilm assays, we tested an FCZ-susceptible and an FCZ-resistant isolate from each species, whenever possible.

Importantly, both F2 and F2.4 inhibited effectively the proliferation of CNA and C. albicans dispersion cells from pre-formed biofilms, and this activity was stronger than that against planktonic cells, for all Candida isolates (i.e., DMIC < PMIC, Table 2). These data suggest that both F2 and F2.4 have promising effect in the control of dissemination and re-infection by dispersion cells from Candida biofilms.

Although some studies reported that dispersion cells have increased virulence and antifungal resistance (Nobile and Johnson, 2015), in our work dispersion cells did not have decreased antifungal susceptibility to AMB, MCZ and FCZ compared with planktonic cells (Table 2), in agreement with that reported by Vila et al. (Vila and Rozental, 2016). Notable exceptions were the dispersion cells from C. glabrata biofilms, which had increased susceptibility to FCZ compared with planktonic cells, and the dispersion cells from C. albicans IAL-40 and C. tropicalis ATCC 200956, which were more susceptible to MCZ than planktonic cells (Table 2).

Previous studies reported limited, albeit significant, antibiofilm effect of S. adstringens fraction F2 on C. tropicalis biofilms (29% inhibition in biofilm formation by treatment with 4xPMIC) (Morey et al., 2016). However, in our work, F2 and F2.4 (and the fungistatic antifungals MCZ and FCZ) could not inhibit the metabolic activity of cells from biofilms under formation, or sessile cells from established Candida biofilms at the concentrations tested (Table S2). Only AMB inhibited biofilm formation and pre-formed biofilm sessile cells (particularly for C. parapsilosis isolates), at concentrations ranging from 0.5 to >16 mg/L (Table S2). This discrepancy of anti-biofilm activity results for F2 and F2.4 could be explained by the difference between the fungal suspension concentrations used in the in vitro biofilm models (10^3 CFU/mL used here, versus 10^4 CFU/mL used by Morey et al. (Morey et al., 2016)), indicating that initial fungal concentration may directly affect the anti-biofilm activity of test compounds. On the other hand, the antifungal activities of F2 and F2.4 on C. albicans ATCC 10231 biofilms under formation determined using our protocol (data not shown) were similar to those reported by Luiz et al. (Luiz et al., 2015).
3.3. A vaginal gel containing *S. adstringens* proanthocyanidin polymeric tannins reduces vaginal candidiasis in vivo

Interestingly, F2 and F2.4 had similar antifungal effects against planktonic and biofilm dispersion cells, although subfraction F2.4 represents a semi-purified fraction from F2 obtained by Sephadex LH-20 column chromatography (Ishida et al., 2006). Nevertheless, the final tannins extraction yield for F2 was approximately 28% versus 15% for F2.4 obtained from *S. adstringens* stem bark (Ishida et al., 2006). Considering that F2 and F2.4 had similar antifungal activities, but F2.4 production was more laborious and costly, we chose fraction F2 for incorporation in a vaginal gel formulation to be tested in vivo.

To evaluate if *S. adstringens* tannins had potential in the treatment of vaginal candidiasis, we induced this disease in mice by vaginal inoculation of either *C. glabrata* ATCC 2001 or *C. albicans* ATCC 10231 yeasts, the two main *Candida* species involved in the VVC and RVVC, and then treated infected mice with a vaginal gel containing two different concentrations of F2 (2.5% and 5%), or 5% F2 combined with oral FCZ (20 mg/kg). As a comparison, mice were kept untreated (untreated group), or were treated with the vaginal gel only (vehicle control) or with standard antifungals alone (20 mg/kg FCZ alone or topical 2% MCZ).

Vaginal candidiasis by *Candida glabrata* ATCC 2001 was more susceptible than that by *C. albicans* ATCC 10231 to the therapeutic protocols tested here (Fig. 2). The vaginal gel containing fraction F2 from *S. adstringens* significantly decreased (by ~100 fold, for 5% F2) the fungal burden in the vaginal tissue of mice infected by *C. albicans* (concentration of 5%, p < 0.001) and by *C. glabrata* (concentrations of 2.5% and 5%, p < 0.01 and p < 0.001, respectively) compared with the untreated group. Oral FCZ and the combination of F2 and FCZ decreased the vaginal fungal burden to a similar extent as topical 5% F2 only (no statistically significant differences, at p < 0.05). However, for *C. albicans* in particular, we observed that the fungal burden values were consistently low in the F2/FCZ combined therapy dataset, with increased variation between data points in the monotherapy with FCZ or 5% F2 (Fig. 2). The vaginal gel formulation containing 2.5% F2 controlled *C. glabrata* fungal burden to the same extent as 2% MCZ, which had antifungal efficacy only against *C. glabrata* infection (p < 0.001). Treatment with the drug-free gel-base formulation (F2 vehicle) did not decrease the fungal burden in mouse vaginal tissue (Fig. 2).

Histopathological analysis showed that untreated mice infected with *C. albicans* ATCC 10231 or *C. glabrata* ATCC 2001 had fungal invasion in the vaginal mucosa and higher fungal burden into the vaginal canal (score = + 3 to + 4) (black arrows in Fig. 3, C and E). In contrast, treatment with the vaginal gel containing 5% F2 reduced the fungal burden in the vaginal tissue and vaginal canal of infected animals (score = +1 to +2), corroborating the CFU count data (Fig. 3, D and F).

Fig. 3. Effect of a gel formulation containing 5% of fraction F2 from *Stryphnodendron adstringens* on the histopathology of vaginal tissue from BALB/c mice. (A, B) Uninfected mice kept untreated (A) or treated with F2 (B). (C, D) Animals infected with *Candida albicans* ATCC 10231 and kept untreated (C) or treated with F2 (D). (E, F) Animals infected with *Candida glabrata* ATCC 2001 and kept untreated (E) or treated with F2 (F). Black arrows indicate the presence of fungi. Scale bars = 50 µm.
The histopathological analysis also suggested that the gel formulation containing 5% F2 caused no histological alterations on the unin­
jected vaginal mucosa of BALB/c mice (Fig. 3A-B). These data indicate that fraction F2 does not cause topical toxicity, and confirm previously published data showing that both F2 and F2.4 have low cytotoxicity in vitro (towards mammalian epithelial cells, macrophages and red blood cells) (Ishida et al., 2006), and in vivo, when administered orally to rodents (lethal dose of 50%, of 2699 mg/kg) (Rebecca et al., 2002), and no genotoxic effects at doses of up to 2250 mg/kg F2 in Swiss rats (Costa et al., 2010).

4. Conclusions

The results of in vitro and in vivo testing in a vaginal candidiasis model show that proanthocyanidin polymeric tannins obtained from S. adstringens stem bark have potential for use in the safe topical treatment of vaginal candidiasis, corroborating the popular use of “barbatimão” stem bark extracts in the treatment of vaginal infections (Brasil, 2014, 2010; Santos et al., 1987). Topical treatment with proanthocyanidin polymeric tannins from S. adstringens stem barks may be an interesting alternative to control vaginal infections by Candida isolates susceptible and resistant to current antifungals, and may provide particularly effective control of Candida dissemination and re-infection, given that these tannins have activity against dispersion cells from biofilms. In addition to their antifungal effects, the anti-inflammatory activity of the proanthocyanidin polymeric tannins present in fraction F2 may contribute to reduce the signs and symptoms of vaginal candidiasis in patients.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jep.2018.01.008.

References