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Full Length Research Paper

Experimental vaginal candidiasis: Assessment of *Origanum vulgare* for its treatment

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The aim of this work was evaluate action of oregano (*Origanum vulgare*) essential oil in treatment of experimental vaginal candidiasis. A batch of 50 Wistar rats was randomly allocated into four experimental groups corresponding to treatments: T1, 1.5% oil; T2, 3% oil; T3, Nystatin and T4, control treatment (emulsion). Oil concentrations were emulsified in agar suspension at 0.8%. Vulvovaginal candidiasis was established in ovariectomized and estrus-induced rats by intravaginal inoculation of *Candida albicans* (10^6 cells-ml). Experimental rats were treated intravaginally daily for a period of 30 days and tested for clinical and hematological parameters, agent retro isolation and macroscopic alterations. Rats from T4 (Negative) treatments did exhibit major alterations exhibited in clinical parameters major alterations. Macroscopic lesions were evident in animals T1 and T4, e.g. erythema, white spots and vaginal mucosa ulcerations; two rats from T4 and one from T1 showed an enhanced uterine volume. Colony counts (UFCs) at the end of experimental period were of 3.1, 2 and 6.5 $\log^2$ for T1, T2 and T4, respectively. T3 showed the lowest UFC value of 0.5 log. Experimental results, considered as preliminary, showed a good performance for 3% *O. vulgare* essential oil formulation on the control of experimental vaginal candidiasis.

Key words: Vaginal candidiasis, essential oil, *Origanum*.

INTRODUCTION

Candida is a pleomorphic organism capable of taking the form of yeast, mycelium or hypha, and displaying the characteristic of pseudo mycelium under specific nutritional environments. These yeasts can become pathogenic when local and host-related factors favor the increase of cells, leading to occurrence of different clinical conditions (Cleff et al., 2007; Chami et al., 2004; Colombo and Guimarães, 2003; Pressler et al., 2003)

Colonization of the vaginal mucosa by *Candida* spp can lead to candidiasis vulvovaginal (CVV), which is a public health concern (Colombo and Guimarães, 2003; Nurbhai et al., 2001; Patel et al., 2004; Pressler et al., 2003; Sobel, 1985; Val and Filho, 2001; Ziarrusta, 2002).

Conventional CVV treatment is based on antifungals from polyenic class, mainly drugs such as Nystatin and azole (Rex et al., 2000; Patel et al., 2004; Sobel, 1985) However, recent studies have indicated that *Candida albicans* resistance to azole antifungals may lead to treatment failure (Cernicka and Subik, 2006; Cleff et al., 2007; Patel et al., 2004; Silva et al., 2002; Sobel, 1985). The use of essential oils in the treatment of fungi relevant to veterinary medicine has proven promising *in vitro* and *in vivo* experiments, especially in the case of *Origanum vulgare* (Cleff et al., 2010; Foldvari et al., 2000; Giordani et al., 2004). Antimicrobial action of *O. vulgare* has been

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attributed to phenolic compounds that are major components of the essential oil, e.g. monoterpenes sesquiterpenes (D'Antuono, et al., 2000; García and Sanz, 2001; Johnson, 2004; Rodrigues et al., 2004; Russo et al., 1998). The focus of this work was to evaluate action of oregano (*O. vulgare*) essential oil in treatment of experimental vaginal candidiasis in rats.

MATERIALS AND METHODS

The essential oil was extracted during a period of four hours prior to hydrodistilation using a modified Clevenger-type apparatus, according to Brazilian Pharmacopeia IV (1988) and analyzed in a gas chromatograph with a flame ionization detector (GC/FID, Schimadzu 17A model). An analytical terpene standard solution was prepared for 100 µg L^{-1} in hexane and adjusted to meet oil condition.

In vivo research work 50 female Wistar albino rats (*Rattus novergicus*), with an average weight of 240 g were used. The animals were housed at the Central Bioterium, Federal University of Pelotas (UFPel), and maintained under controlled temperature, humidity and photoperiod conditions. Diets were supplied according to body weight and water was *ad-libitum*. All experimental animals were subjected to a 2-week period of adaptation to handling by researchers, strictly following principles established in Ethics in Animal Experimentation, as abided by the Brazilian College of Animal Experimentation.

All females were ovariectomized and treated weekly to estradiol valerate (induction dose: 10 mg kg^{-1} Sc^{-1}; maintenance dose: 4 mg kg^{-1} Sc^{-1}) prior to inoculation and immune suppressed with 2 mg L^{-1} dexamethasone (Fortecortin, Laboratório Merk) added to drinking water (Foldvari et al., 2000; Martinez et al., 2001; Nurbhai et al., 2001).

Yeast inoculum was prepared by growing isolated *C. albicans* for 24 h at 37°C suspended in Phosphate buffer saline (PBS) and homogenized and adjusted to 10^6 CFU mL^{-1}. Prior to inoculation, specimens from vaginal cavity were collected to verify presence of yeasts from genus *Candida*. Once adaptation period was completed all animals were intravaginally inoculated with 0.2 ml inoculum suspension using a 1 ml scale syringe. The presence of *C. albicans* in the vaginal lavage fluid was assessed through the swab culture, and when positive it was held as evidence of symptomatic infection.

The female rats were randomly assigned to four experimental groups as follows: T1 (n=15), 0.2 ml suspension at 1.5% oil; T2 (n=15), 0.2 ml suspension at 3.0% oil; T3 (n=10), 0.2 ml nystatin suspension (1000 UI d-1); T4 (n=10), 0.2 ml 0.8% agar suspension. Animal general conditions was monitored throughout the experiment. The following parameters were monitored: Apathy, dehydration, skin lesions, hair loss, hair chills, spots or reddish coloration on the vaginal mucosa, presence secretion and occurrence of death. All animals were weighed on a weekly basis.

Autopsies were performed along and at the end of experiment, involving collection of reproductive tract and internal organs such as stomach, intestine, spleen, liver and kidneys, all of which were subjected to mycological and histopathological analyses. The tissues from these organs were cultured "Pour platte" on Petri dishes containing Sabouraud Dextrose Agar (Merck, Germany) and maintained at 37°C to perform count, macromorphology and micromorphology characterizations of *C. albicans*.

RESULTS AND DISCUSSION

The essential oil used in treatment of animals was extracted according to methodology duly established (Sobel, 1985) and its fraction composition was as follows: α-phellandrene (2.47%), α-terpinene (2.83%), limonene (3.60%), linalool (2.89%), γ-terpinene (2.83%), 4-terpineol (7.57%), thymol (8.42%), carvacrol (9.44%) and β-caryophyllene (2.92%). These results are in agreement with data reported in literature in which composition of essential oil of *O. vulgare* has been shown to contain a phenol range between 80.2 and 98%, being 4-terpineol, carvacrol, thymol and γ-terpinene the most conspicuous. Concentration and proportion of these different components are key to product efficiency, since previous works have shown that isolated compounds such as thymol and carvacrol do not achieve same efficacy observed when essential oil was applied (Colombo and Guimarães, 2003; Sobel, 1985; Val and Filho, 2001).

The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) in front of isolated essential oil used for inoculation of experimental animals were determined by microdilution in broth (NCCLS M27-A2), and values of MIC were 1.2 and 2.5 µl mL^{-1} for MFC. Treatment efficacy was determined through clinical and hematological parameters, microbio-logical evaluation and macroscopic and histopathological alterations.

It was observed that animals from group T4 (Negative control) exhibited more frequent alterations on the clinical parameters evaluated when compared to the other experimental groups (T1, T2 and T3). Animals from group T3 (Positive control) showed lowest percentage values for alterations on these parameters (Figure 1).

Animal's weight did not change throughout experiment. Weight loss was recorded across all experimental groups during second week, which can be explained by the stress underwent by female rats as result of changes in their routine and daily handling for administration of pharmaceuticals.

Hemogram tests performed on animals from groups T1, T2 and T3 yielded results within the expected physiological parameters for the species under study (Diniz et al., 2006); no cell type count difference among experimental groups was found. However, animals in T4 showed values for hematocrites, hemoglobin and hematia below normal physiological standards, thus constituting a case for anemia; four animals exhibited enhanced PPT values, possibly due to dehydration. Leucogram test results indicated a decrease in total leukocytes across all experimental groups caused by lymphopenia, which can be explained by corticoid administration necessary for development of experi-mental candidiasis (Martinez et al., 2001). Chami et al. (2004) working on a model for experimental candidiasis found that in the immune suppressed group of rats only one in seven animals was infected with *C. albicans*, thus demonstrating the requirement for immune suppression in a successful model.

Research data on lymphocyte values in Wistar rats

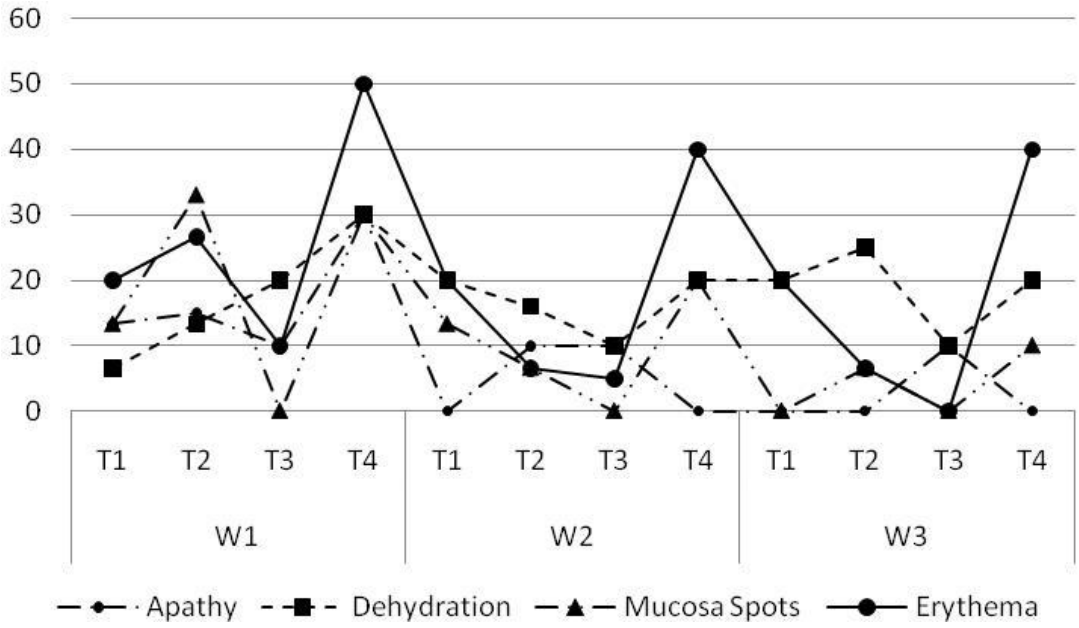

Figure 1. Percentage values for clinical alterations observed for a period of 30 days in experimental rats with vaginal candidiasis. *Evaluation carried out for a period of 3 weeks (W1, W2, W3), in all three observations per animal for each experimental group; T1: *O. vulgare* 1.5%; T2: *O.vulgare* 3%; T3: positive control; T4: negative control.

have yielded values between 5.6 × 10 µl to 8.3 × 10 µl, while values for total leukocytes are in the range of 6 × 10 µl to 10 × 10 µl (Cernicka and Subik, 2006). In group T4, five rats showed notorious leukopenia by lymphopenia, from which four animals presented total leukocyte count between 3000 and 4100 mm^{-3} and one animal with total leukocyte count of 1900 mm^{-3}.

The T4 results may be consequence of maintaining levels of vaginal infection by *C. albicans* coupled with animal's immune suppression, which most likely caused systemic alterations reflected on hemogram test results. In practice, one known factor that can enhance vaginal infection by *C. albicans* is a host weak condition, be it the result of immune suppressive diseases or chronic corticoid drug use (Colombo and Guimarães, 2003; Martinez et al., 2001; Pressler et al., 2003; Sobel, 1985). According to data reported in the literature, blood cell structures are prone to alterations during different disease phases (Diniz et al., 2006).

Macroscopic alterations from reproductive tract observed during autopsy occurred in some, but not all, animals, being more conspicuous on the vaginal mucosa and appearing as erythems, white spots and ulcerations in animals from groups T2 and T4. Two animals from group T4 and one from group T1 exhibited an increase in uterine volume, while no alterations whatsoever were observed in animals from group T3 (Figures 2 and 3).

The observed lesions were in agreement with the description given for vulvovaginal candidiasis (Martinez et al., 2001; Ziarrusta, 2002) being that progression of this disease involves epithelial invasion with release of substances such as prostaglandins and bradicinins, responsible for inducing local inflammatory processes leading to edema, erythems and increased discharge (Sobel, 1985). Several works have shown that vaginal infection by *C. albicans* can be influenced by hormone levels, being enhanced by high levels of estrogen and progesterone, the former enhancing glycogen accumulation in the vagina's epithelial cells which in turn increases the carbohydrate source to sustain growth and germination of *Candida* (Sobel, 1985; Val and Filho, 2001; Ziarrusta, 2002).

The agent retro isolation was obtained from vaginal mucosa tissue, showing presence of yeasts in groups T1 (4.5 log UFC) and T2 (2 log UFC); however, the number of units forming colonies (UFC) was reduced when compared to T4 (6.5 log UFC). A reduction in number of *Candida* colonies across treatments became evident; however, yeast was not completely eliminated after a 30-day treatment application period. Results for animals in T2 were close to those observed for individuals in T3 (e.g. 0.5 log UFC), were yeast count after 30 day treatment application period was practically zero (Table 1).

Nystatin (T3) proved an effective treatment against experimental vaginal candidiasis, which is in agreement with previous works and with its appointment as a therapeutic pharmaceutical (Cernicka and Subik, 2006; Rex et al., 2000; Patel, 2004). In our work only T2 came close to the result obtained with the use of conventional

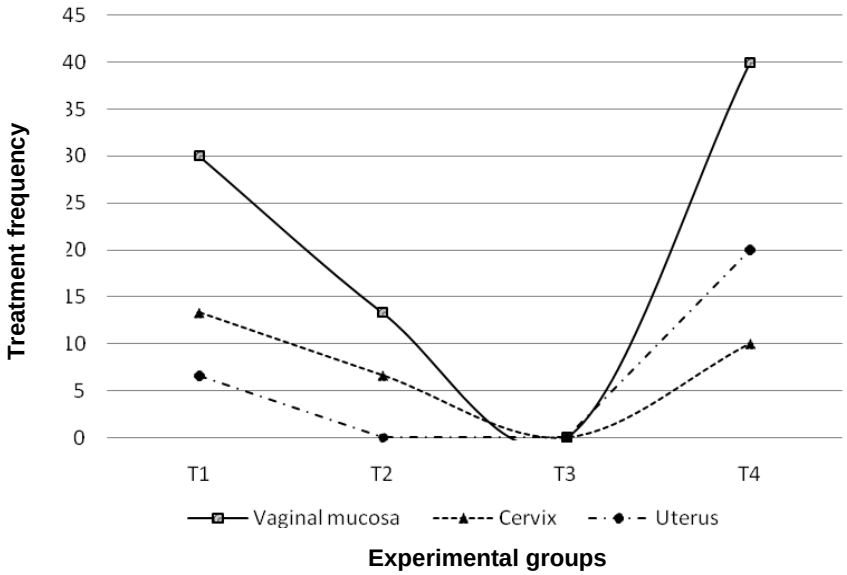

Figure 2. Treatment frequency for animals showing reproductive tract macroscopic lesions as determined through post mortem examination of rats euthanized after inoculation with *C. albicans.* T1, T2, T3 and T4 experimental groups.

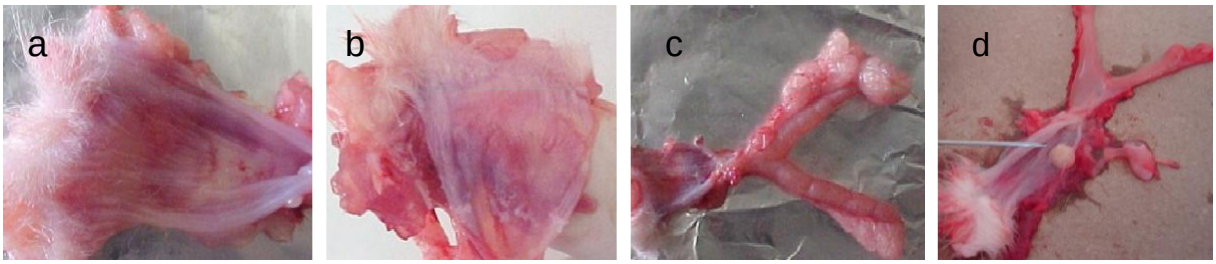

Figure 3. Vaginal mucosa macroscopic alterations (a, b), enhanced uterine volume (c) and fungus colony established at the cervix entry (d), as exhibited by experimental animals from group T4 (Negative control).

Table 1. Microbiological study on therapeutic efficacy in rats of essential oil from *Origanum vulgare* versus nystatin on the control of vaginal candidiasis.

Treatments	Day 15	Day 30	Day 30
	Log2CFU/sample Mean±SD	Log2CFU/sample Mean±SD	Percentage retro isolation
O. vulgare 1.5% (T1)	6.33±1.5	4.5±0.7	33%(5/15)[a,b]
O. vulgare 3% (T2)	5.66±1.1	2±2.8	27%(4/15)[a]
Nystatin (T3)	2.51±2.6	0.5±0.7	20%(2/10)[a]
Positive control (T4)	5.66±1.25	6.5±0.7	50%(5/10)[b]

Isolation of *C. albicans* in vaginal mucosa of rats after 15 days and 30 days of treatment with *O. vulgare* 1.5% (T1), *O. vulgare* 3% (T2), Nystatin (T3) and respective diluents (T4). *P<0.05 compared to control treatment.

antifungals (T3), which differs with results published elsewhere (Foldvari et al., 2000).

Conclusions

The results produced by this work stress the need for further evaluations, especially those related to histopathological analyses, since they provide the kind of data necessary to the elucidation of oregano oil treatment efficacy. The experimental results, considered as preliminary, showed a good performance for the 3% *O. vulgare* essential oil formulation on control of experimental

vaginal candidiasis.

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