Short communication

Alternative drugs against Trichomonas vaginalis

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الأدوية البديلة المضادة للمشعَّرة المهبلية

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الخلاصة: للتعرُّف على أثر الأدوية الأخرى غير المترونيدازول، عولجت ثلاث حوامل مصابات بالعدوى بالمشعَّرة المهبلية بالدوكسي سيكلين بجرعتين يومياً مقدار كل منها 200 مغ ولمدة أسبوع، فيما عولجت ثلاث حواملٍ أخريات بالبرازيكوانتيل، بجرعة وحيدة مقدارها 40 مغ/كغ من وزن الجسم. ولم يكشف أيٌّ تأثير علاجي لأيٍّ من الدواءَيْن. أما في التجارب المختبرية، فقد أدى الأوكسي تتراسيكلين إلى موت المشعَّرة المهبلية بتركيز 15 مغ في 0.5 مل من المستنبت، كما أدت خلاصة شجر الآس الشائع إلى موت المشعَّرات المهبلية في درجة باهاء pH 4.65 ولكنها فشلت في قتلها في درجة باهاء pH 6.00. كما سببت خلاصة اليوكاليبتوس الكومالديني بتركيز 50 مغ في 0.1 مل من المستنبت وفي درجة باهاء pH 5.35 إلى موت المشعَّرات المهبلية بعد 24 ساعة.

ABSTRACT To investigate the effect of drugs other than metronidazole, 3 non-pregnant women infected with Trichomonas vaginalis were treated with doxycycline, 2 × 200 mg/day for 1 week. Another 3 women were treated with praziquantel, single dose, 40 mg/kg body weight. No therapeutic effect was detected for either drug. In vitro, oxytetracycline led to death of T. vaginalis at a concentration of 15 mg in 0.5 mL medium. Extract of Myrtus communis caused death of T. vaginalis at pH 4.65, but failed to do so at pH 6.00. Extract of Eucalyptus comaldensis (50 mg in 0.1 mL medium) at pH 5.35 caused death of T. vaginalis after 24 hours.

Traitements alternatifs contre Trichomonas vaginalis

RÉSUMÉ Afin d'examiner l'effet d'autres médicaments que le métronidazole, 3 femmes non enceintes infectées par Trichomonas vaginalis ont été traitées à la doxycycline, 2 x 200 mg/jour pendant une semaine. Trois autres femmes ont été traitées au praziquantel en dose unique de 40 mg/kg de poids corporel. Aucun effet thérapeutique n'a été détecté pour ces deux médicaments. In vitro, l'oxytétracycline a entraîné la mort de T. vaginalis à une concentration de 15 mg dans 0,5 mL de milieu. Un extrait de Myrtus communis a causé la mort de T. vaginalis à un pH de 4,65 mais pas à un pH de 6,00. Un extrait d'Eucalyptus comaldensis (50 mg dans 0,1 mL de milieu) à un pH de 5,35 a entraîné la mort de T. vaginalis après 24 heures.

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Introduction

Trichomonas vaginalis is a flagellate protozoan which infects the urogenital tract of men and women [*1*]. It is transmitted by sexual intercourse [*2,3*], by non-venereal means such as sharing of contaminated towels or underclothing, or the use of non-sterile medical examination tools [*2,3*]. In women it causes vaginitis and cystitis and in men it causes urethritis and prostatitis [*4*].

Metronidazole is the mainstay of treatment for *T. vaginalis* infection and can be given as a single dose, 2 g orally (divided into 1 g in the morning and 1 g at night), or 250 mg 3 times/day for 7 days [*5,6*].

Appropriate treatment with metronidazole cures 60%–80% of cases with a single dose, but the cure rate increases to > 90% when sexual partners, who are usually asymptomatic, are treated simultaneously to prevent re-infection [*7*].

The chemotherapeutic trial presented in this study is the first attempt in Iraq to try to identify alternatives to metronidazole, the only currently available treatment for trichomoniasis. Under the circumstances of economic sanctions and shortage of drugs, it is essential to carry out therapeutic investigations on chemical and plant sources.

Tetracycline has an antiprotozoal effect against *Dientamoeba fragilis* and *Balantidium coli* [*9*]; therefore, tetracycline derivatives doxycycline and oxytetracycline were chosen for this investigation. Both are readily available, the treatment course is short, resistance has not been recorded and they do not have the bitter taste that is associated with metronidazole. In addition, oxytetracycline is the drug of choice in the treatment of *T. foetus* in cattle (veterinary use) [*10,11*]. The drug oxytetracycline and plant extracts of *Myrtus communis* and *Eucalyptus camaldulensis* were chosen for *in vitro* investigation because they have antiparasitic activity [*10–12*].

Methods

Samples were obtained from the vaginal discharges of infected women by sterile vaginal swab and cultured in selective medium, Bacto trichomonas broth, (Difco No. 0911.02, Difco Laboratories, Michigan), incubated at 37 °C and checked for the presence of *T. vaginalis* after 48–72 hours. This was done by taking a drop from the bottom of the culture using a sterile Pasteur pipette, transferring to a slide and examining under the high power objective for ≥ 10 minutes.

Two drugs were tested experimentally *in vivo*, and 1 drug and 2 plant extracts *in vitro*, in order to study their effect on *T. vaginalis*.

In vivo

This study was carried out in the first 6 months of 2001 in Basra Maternity Hospital, the main centre for gynaecology and obstetrics in the city. Diagnosis of *T. vaginalis* infection was made by 2 of the authors by wet preparation and *in vitro* cultivation. All 7 non-pregnant, infected women presenting during the study period were informed about the study and all but 1 agreed to participate in the *in vivo* study. The age range of the women was 23–45 years.

Two drugs were used, doxycycline and praziquantel. The diagnosis and treatment outcome were assessed by wet preparation microscopy and culture methods [*3*].

Three non-pregnant patients infected with *T. vaginalis* were treated orally with doxycycline 200 mg (Vidipha, Ho Chi Minh City, Socialist Republic of Viet Nam), 2 capsules/day for 1 week.

A second group of 3 non-pregnant patients infected with *T. vaginalis* were

treated with praziquantel (Shin Poong Pharmaceutical Company Ltd., Seoul, Republic of Korea), 40 mg/kg body weight in a single dose divided into 2 parts taken 5 hours apart. Re-examination was done on the 3rd and the 5th days after completing treatment.

In vitro

The antibiotic oxytetracycline, 100 mg/mL preparation (Ciba-Geigy Ltd., Basle, Switzerland), was added to tubes containing 0.5 mL Bacto trichomonas broth in the following amounts: 0.1 mL, 0.15 mL, 0.2 mL and 0.5 mL. The mixtures were used on the same day they were made up. They were inoculated with *T. vaginalis* (5×10^6 to 10×10^6 organisms/mL) and incubated at 37 °C. The tubes were examined for *T. vaginalis* activity immediately and over a 24-hour period.

Plant material and extraction

Two plants, *M. communis* L. (Myrtaceae) and *E. camaldulensis* L. (Myrtaceae) were investigated. Both of these are grown in Basra. Extraction was carried out according to the method described in Harborne [*8*]. Briefly:

- leaves were separated from other parts of the plant and washed with water;
- the leaves were then dried under sunlight with good ventilation and then ground finely in a mill;
- 10 g of the ground leaves was mixed with 100 mL of distilled water in a flask and heated on a magnetic stirrer at 40 °C–50 °C for 4 hours/day for 12 days;
- the suspension was then centrifuged at 6000 rpm for 30 minutes;
- the supernatant was decanted and clarified by filtration through a sterile filter paper (0.45 μ) then made up to 100 mL with distilled water.

The pH of the *M. communis* extract was 4.65 and that of the *E. camaldulensis* extract 5.35. The liquid plant extracts were tested at different concentrations with *T. vaginalis*, 10^6 organisms/mL.

Myrtus communis

Tubes were prepared with 0.1 mL of medium (Bacto trichomonas broth). Plant extract was added in the following amounts: 0.1 mL, 0.2 mL, 0.5 mL and 1.0 mL. Control tubes were prepared in a similar manner, omitting the extract. Since *T. vaginalis* is not viable at pH < 4.9, the pH of the extract (4.65) was adjusted to 6.00 with 10% NaOH, and a second series of tubes prepared. All samples were examined by the same investigator at 0.0 hours, 0.5 hours, 1.0 hour and 24.0 hours.

Eucalyptus camaldulensis

Test and control tubes were prepared and examined in a similar manner. Plant extract was added in the following amounts: 0.2 mL, 0.5 mL, 1.0 mL and 2.0 mL.

Results

In vivo

In the 3 women who were treated with doxycycline, *T. vaginalis* was still alive and active when they were re-examined after treatment.

Similarly, praziquantel had no effect on *T. vaginalis* infection.

In vitro

Oxytetracycline caused death of *T. vaginalis* immediately in the tubes which had 0.15 mL, 0.2 mL and 0.5 mL of the drug in

Table 1 Effect of oxytetracycline on viability of Trichomonas vaginalis examined immediately after inoculation

Oxytetracycline, amount added to 0.5 mL medium	Viability of T. vaginalis
0.0 mL (control)	Alive
0.1 mL (10 mg)	Active flagellae
0.15 mL (15 mg)	Dead
0.2 mL (20 mg)	Dead
0.5 mL (50 mg)	Dead

Alive = normal activity and movement of the organism and the flagellae.
Active flagellae = flagellae only moving.
Dead = no normal activity at all.

0.5 mL medium. Flagellae remained active only in the tube with 0.1 mL oxytetracycline in 0.5 mL medium (Table 1).

Aqueous extract (pH 4.65) of *M. communis* showed an effect against *T. vaginalis* (Table 2): the organism was dead immediately in the tubes containing 50 mg and 100 mg extract, and within 0.5 hours in the tube with 20 mg extract. At pH 6.00, however, *M. communis* extract had no effect against the organism (Table 3).

Aqueous extract (pH 5.35) of *E. camaldulensis* showed some effect on viability of *T. vaginalis*. After 24 hours, *T. vaginalis* was dead in the tubes containing 50 mg, 100 mg and 200 mg aqueous plant extract (Table 4).

Discussion

Interestingly, this study has shown that although doxycycline had no effect on *T. vaginalis* infection *in vivo*, oxytetracycline had a lethal effect *in vitro*. Since this drug is available in several countries other than Iraq as a broad spectrum antibiotic in human medicine [*12*], it is possible it could, after careful evaluation, be used as an alternative drug if it was available in the country, especially among women who cannot tolerate the bitter taste of metronidazole and those having resistant infection.

Even though praziquantel has a good therapeutic effect on the intestinal flagellate *Giardia lamblia* and the intestinal amoeba *Entamoeba histolytica* [*13*], no effect was observed in our study against the urogenital flagellate *T. vaginalis*.

Table 2 Effect of Myrtus communis extract (pH 4.65) on viability of Trichomonas vaginalis (in vitro)

Amount of extract added 0.1 mL medium	Viability of T. vaginalis in 0.1 mL medium Incubation period (hours) 0.0	0.5	1.0	24.0
0.0 mL (control)	Alive	Alive	Alive	Alive
0.1 mL (10 mg)	Active flagellae	Active flagellae	Active flagellae	Dead
0.2 mL (20 mg)	Active flagellae	Dead	Dead	Dead
0.5 mL (50 mg)	Dead	Dead	Dead	Dead
1.0 mL (100 mg)	Dead	Dead	Dead	Dead

Alive = normal activity and movement of the organism and the flagellae.
Active flagellae = flagellae only moving.
Dead = no normal activity at all.

Table 3 Effect of Myrtus communis extract (pH 6.00) on viability of Trichomonas vaginalis (in vitro)

Amount of extract added to 0.1 mL medium	Viability of T. vaginalis Incubation period (hours) 0	0.5	1	24
0.0 mL (control)	Alive	Alive	Alive	Alive
0.1 mL (10 mg)	Alive	Alive	Alive	Alive
0.2 mL (20 mg)	Alive	Alive	Alive	Alive
0.4 mL (40 mg)	Alive	Alive	Alive	Alive
0.5 mL (50 mg)	Alive	Alive	Alive	Alive
1.0 mL (100 mg)	Alive	Alive	Alive	Active flagellae
1.5 mL (150 mg)	Alive	Active flagellae	Active flagellae	Active flagellae
2.0 mL (200 mg)	Alive	Active flagellae	Active flagellae	Active flagellae
3.0 mL (300 mg)	Alive	Active flagellae	Active flagellae	Active flagellae

Alive = normal activity and movement of the organism and the flagellae.
Active flagellae = flagellae only moving.

As part of this study, 2 plants were investigated for the first time because they are known to have some antibacterial and antiparasitic activity [*14–17*]. We found that *M. communis* extract killed the organism at pH 4.65, but no such effect was observed at pH 6.00 (the optimal pH for growth of *T. vaginalis*).

Table 4 Effect of Eucalyptus camaldulensis extract (pH 5.35) on viability of Trichomonas vaginalis (in vitro)

Amount of extract added to 0.1 mL medium	Viability of T. vaginalis in 0.1 mL medium Incubation period (hours) 0	0.5	1	24
0.0 mL (control)	Alive	Alive	Alive	Alive
0.2 mL (20 mg)	Active flagellae	Active flagellae	Active flagellae	Less active flagellae
0.5 mL (50 mg)	Active flagellae	Active flagellae	Less active flagellae	Dead
1.0 mL (100 mg)	Active flagellae	Less active flagellae	Less active flagellae	Dead
2.0 mL (200 mg)	Active flagellae	Less active flagellae	Less active flagellae	Dead

Alive = normal activity and movement of the organism and the flagellae.
Active flagellae = flagellae only moving.
Dead = no normal activity at all.

Promising results were obtained with *E. camaldulensis* extract. Consequently, this plant extract needs further investigation. Research into other chemical and plant sources to look for alternative drugs against *T. vaginalis* is essential. The characteristics required of such drugs are: inexpensive; available in the country; not having a bitter taste (cf. metronidazole); no parasite resistance (cf. metronidazole); safe to use in pregnancy; and short treatment course.

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