(45) Date of publication and mention of the grant of the patent: 20.04.2016 Bulletin 2016/16
(21) Application number: 09746834.2
(22) Date of filing: 05.05.2009
(54) CONIFER GREEN NEEDLE COMPLEX FOR USE IN TREATING A TRICHOMONIASIS INFECTION OF THE UROGENITAL TRACT.
GRÜNNADELCOMPLEX DES Nadelbaums für die Verwendung in der Behandlung von Trichomoniasis Infektion des Urogenitaltraktes.
CONIFÈRES COMPLEX AIGUILLE VERTE POUR L’UTILISATION DANS LE TRAITEMENT DE L’INFECTION PAR TRICHOME NASE DU TRACTUS UROGÉNITAL
(84) Designated Contracting States: AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO SE SI SK TR
(30) Priority: 14.05.2008 RU 2008119139
(43) Date of publication of application: 06.04.2011 Bulletin 2011/14
(73) Proprietors:
• Roschin, Viktor Ivanovich St.Petersburg 197220 (RU)
• SULTANOV, Vagif Sultanovich St.Petersburg, 195213 (RU)
(72) Inventors:
• ZHEBRUN, Anatolii Borisovich St.Petersburg 190000 (RU)
• NIKITINA, Tamara Valentinovna St.Petersburg 194223 (RU)
• KULIGASHOVA, Lidija Borisovna St.Petersburg 198259 (RU)
• BEREZINA, Ludmila Aleksandrovna St.Petersburg 197373 (RU)
• ROSCHIN, Viktor Ivanovich St. Petersburg 197220 (RU)
• SULTANOV, Vagif Sultanovich St. Petersburg, 195213 (RU)
(74) Representative: Ellmeyer, Wolfgang et al Häupl & Ellmeyer KG Patentanwaltskanzlei Mariahilfer Strasse 50 1070 Wien (AT)
(56) References cited:
• RU-C1- 2 021 803 RU-C1- 2 272 626 RU-C2- 2 208 950
The invention belongs in the field of medicine and can be used in the treatment of acute and chronic and asymptomatic trichomonas infections e.g. infections of the urogenital tract.

Description

Technological field

[0001] The invention belongs in the field of medicine and can be used in the treatment of acute and chronic and asymptomatic trichomonas infections e.g. infections of the urogenital tract.

Level of technological invention

[0002] Trichomonia is one of the most common sexually transmitted diseases of the urogenital tract. This infection has important medical and socio-economic significance because of the large numbers of people infected, often with a chronic form of the disease and relapses, and the caused damage to different organs and systems. World Health Organisation (WHO) data reveals that at the turn of the 20th and 21st centuries, almost half of all incidences of sexually transmitted infections were of trichomoniasis (Grodstein F. Relation of tubal infertility to a history of sexually transmitted diseases / F. Grodstein, M.B. Goldman, D.W. Cramer // Am. J. Epidemiol. - 1993. - Vol. 137. - P. 577-584; Vilkki M., Pukkala E., Niimen P. et al. Gynaecological infections as risk determinants of subsequent cervical neoplasia//Acta Oncol.-2000. -vol. P. 71-75; Tikhomirov A.L. Urogenital trichomonia, / Tikhomirov A.L., Oleinik Ch.G. // Works of MGMSU - M., 2003, pp. 1-7). Urogenital trichomonia is usually transmitted during sexual contact between infected people or asymptomatic carriers of the disease. Non-sexual transmission can also occur. For example, mothers can infect their children during birth. It can also occur through the reuse of gloves during examinations. In addition, the parasite can survive outside the body on infected items for a few hours, it can be transmitted through contact with infected diapers, bed-pans, toilet seats, and personal hygiene items (Isakov B.A., Zakharkiv Yu.F., Ermenolok D.K., et al. Diagnostics and treatment of urogenital trichomonia. Recommendations for physicians, St-Petersburg, Veliki Novgorod, 2006, p.46).

[0003] A big significance in the epidemic process play people with weakly expressed symptoms and trichomonad carrier that facilitates the epidemiological spreading of the pathogen. Without treatment the protozoon survive in the body and cause numerous complications. Trichomonia has been diagnosed in 70 to 80-year old males who had their last sexual contacts 30 years ago. Some medications, used for the treatment of trichomonas infection, lead to a relative reduction of symptoms. These drugs include acrichine, aminarson, dichloro-diphenyl-trichlorethane, and trichomycin. However, these substances are not very effective.

[0004] Women with urogenital diseases have been treated with phyotherapy that involved using extracts and tinctures obtained from onion, garlic, reddish, horseradish, pine and spruce needles, and jupiter.

[0005] Currently, metronidazole (synthesised in 1959) is widely used for the treatment of urogenital trichomonia. Widspread use was found for combination treatment methods, which included use of peroral and intravaginal drugs such as clion-D, gynalgin, tergynan, and metrogyl (Patent RU 2320319 "Vaginal suppositories", 2006.11.07). However, at present, there is no reliable method of trichomoniasis treatment.

[0006] Lately, treatment of trichomonia has become more difficult because of the appearance of new strains that are multiresistant to therapeutic substances. It has been established that chronic urogenital trichomonia taking place in a form of mixed invasion is a reliable indicator of occurrence of drugs resistant strains of Trichomonas vaginalis.

[0007] The existing treatment regimens often do not provide a significant effect as they do not take into account individual particularities of a patient. For example, the presence of concomitant diseases and changes in the immune system and the biological activity or resistance to therapeutic substances of the strain are not taken into consideration. Assessment of the sensitivity of T. vaginalis to antiprotozoal medications allows for a significant increase in the efficacy of treatment in patients with chronic urogenital trichomonia.

[0008] There are other pharmaceuticals such as mebendazole, butoconazole, and benzoisothiazolin (Vidal, Therapeutic substances in Russia, 6th edition, 2000).


[0010] Traditional anti-protozoal therapy leads to a significant improvement in the patient's condition and etiological recovery. However, clinical recovery does not occur in 64% of patients due to the development of post-trichomonal urethritis (PTU). In these cases, patients complain about unpleasant sensations during urination and periodic mucous-purulent discharge from the urethra (Vilikki M., Pukkala E., Niimen P. et al. Gynecological infections as risk determinants...
of subsequent cervical neoplasia// Acta Oncol.-2000.- vol.- pp.71-75). A thorough laboratory examination for the presence of trichomoniasis produces a negative result and additional anti‐protozoan therapy is not successful. From clinical point of view, post‐trichomonad urethritis has an undulating or monotonous character. Patients become irritable, have sleeping disorders, and can develop sexual dysfunction. The most frequent cause of PTU is the presence of other sexually transmitted pathogens. Urogenital trichomonas provides a depot for the survival of gonococci, fungi, chlamydiae, mycoplasmas, and viruses (Thomason J. L. Trichomonas vaginalis / J.L. Thomason, S.M. Gelbert // Obstetrics and Gynecology. - 1989. - Vol.74. pp.536-541) since enzymes of protozoa cannot always destroy phagocytised by them microorganisms. Subsequently, these pathogens can support the inflammatory process for extended period of time. It has been established that trichomonas prevents the detection of chlamydia in cell culture. Clinically, inflammation due to mycoplasma and chlamydia occurs with relapses, often with damage to the accessory sexual glands. Development of PTU is facilitated not only by microflora that accompanies urogenital trichomoniasis, but also by the formation of L-form microorganisms and the reduction of immunobiological resistance in the host body. This also includes local immunity in the organs of the urinary system.


[0013] Resolving the issue of metronidazole-resistant clinical organisms can be achieved by a number of ways. These include increasing the dose of metronidazole; use of a combination of various anti-trichomonas therapeutic substances, and using these substances In conjunction with nonspecific therapy (Narcisi E.M. In vitro effect of tinidazole and furazolidone on metronidazole-resistant Trichomonas vaginalis / E.M. Narcisi, W.E. Secor // Antimicrob. Agents Chemother. - 1996. - Vol.40. - P. 1121-1125).

[0014] Currently, urogenital trichomoniasis is treated with metronidazole and other nitroimidazoles, such as tinidazole, omedazole, secnidazol, nimorazole, and carnidazole.

[0015] Metronidazole, which is included In individual and combined treatments of trichomoniasis, was selected as the comparator drug for the study described in this document.

The invention

[0016] The present invention is defined by the claims.

[0017] The invention is a new therapeutic substance, of plant origin, that has minimal side effects when used as a treatment for various trichomonal infections.

[0018] The new therapeutic substance is an olive coloured paste. It is composed of a conifer green needle complex (CGNC) and has a coniferous smell and antiprotozoan activity against T. vaginalis (when tested in an in vitro model). CGNC also contains 35-40% water.

[0019] CGNC is an active ingredient in the therapeutic substance, Bioeffective A that is produced in an encapsulated form. It contains chlorophyll derivatives, carotenoids, vitamins A, E, and K, phytosterins, polyphenols, squalene, fatty and acid resin salts, natural antibiotics (phytoncides), essential oils, and other terpenoids (labdanic alcohols, aldehydes, and acids).

[0020] A range of concentrations (100, 200, 300, and 500 mg/ml) of CGNC was used to study the effect on T. vaginalis patient isolates. At a concentration of 100 mg/ml, CGNC exhibited trichomonadocitic and trichomonadostatic properties, and at 300 mg/ml suppressed the growth of T. vaginalis.

[0021] The authors studied the antiprotozoan activity of CGNC against such protozoan as T. vaginalis in a modelled system in vitro.
The results were compared with metronidazole, the medication traditionally used in the treatment of *T. vaginalis*. The parasitic protozoa, *T. vaginalis* was isolated from patients and cultured *in vitro* in nutrient media.

Nutrient medium (4.5 ml) was poured into sterile test tubes and covered with a 5 mm layer of Vaseline to create anaerobic conditions for the growth of trichomonas. Inoculation was carried out using a sterile Pasteur pipette. An aliquot (0.5-1.0 ml) of the test substance was dispensed in the bottom of the test tubes. The samples were incubated at 37°C. Examination of the test tubes to determine the presence of growth was carried out at 48 and 96 hours after inoculation.

A dense, whitish deposit at the bottom of the test tube signified a positive result for trichomonas growth. This was sampled using a Pasteur pipette and prepared for microscopic analysis. The test sample was mixed with a drop of warm isotonic sodium chloride solution or Ringer-Locke solution and placed on a slide. The suspension was covered with a cover glass and observed under the microscopy (x600). The microscope used for this analysis was a MICMED-5 (LOMO, St-Petersburg). The number of protozoan cells in 1 ml of suspension was determined using a Goryaev chamber (as per the guidelines for the chamber).

In this study, a total of 150 people (80 male and 70 female) with inflammatory diseases of the urogenital tract were examined. The patient ages ranged from 17 to 45 years. Thirty isolates of *T vaginalis* were collected from this group. The clinical characteristics and demographics of this group of patients are presented in Table 1.

The study required isolation and selection of *T. vaginalis* isolates that were to be used in the *in vitro* model. Isolates were examined for various characteristics. These included the size and shape of the trichomonas, the pattern of their movement, and their intercellular content. Typically, the trichomonas were motile, pear, and occasionally oval shaped. Their sizes ranged from 13 to 17 μm, and they exhibited an impulse motion. In some cases, movement of the flagella was noticeable under the microscope. The nuclei of the trichomonas were not easily detected in unstained samples. The cytoplasm of trichomonas was generally grainy and vacuolated.

Then, the sensitivity of *T. vaginalis* to metronidazole was examined *in vitro*, using as an indicator the immobilisation of the trichomona by the test-substance. For the sensitivity test, 90% or more of the protozoa were required to be motile. The sensitivity of *T. vaginalis* isolates to metronidazole was determined using serials dilution and the minimum inhibitory concentration (MIC) method. 4.0 ml of the medium was placed in test tubes and mixed with 0.5 ml of solution various concentrations of metronidazole - from 0.25 to 1000 μg /ml, or 1 mg/ml).

Then, 0.5 ml of pathogenic culture containing a known concentration of *T. vaginalis* (cells/ml) was added to the test tubes. Media without the test substance was used as a control. Vaseline was added to the surface (0.5 mm layer) of the tubes to create anaerobic conditions necessary for the growth of *T. vaginalis*. The test tubes were then placed in a thermostat at 37°C. Results were recorded 48 and 96 hours after inoculation.

The sensitivity of trichomonad to metronidazole was determined based on the minimum inhibitory concentration (MIC), which causes immobilisation of all cells of *T. vaginalis*. The strains were considered as resistant to metronidazole, when immobilisation occurred at concentration of metronidazole exceeding 15 μg/ml.

---

**Table 1**

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>Number of patients (% of total)</th>
<th>Age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute diseases of the urogenital tract (female)</td>
<td>9 (6)</td>
<td>19-23</td>
</tr>
<tr>
<td>Acute diseases of the urogenital tract (male)</td>
<td>10 (6.7)</td>
<td>17-26</td>
</tr>
<tr>
<td>Chronic diseases of the urogenital tract (female)</td>
<td>32 (21)</td>
<td>20-35</td>
</tr>
<tr>
<td>Chronic diseases of the urogenital tract (male)</td>
<td>37 (24.7)</td>
<td>20-45</td>
</tr>
<tr>
<td>Sterility (female)</td>
<td>29 (19.3)</td>
<td>28-35</td>
</tr>
<tr>
<td>Sterility (male)</td>
<td>33 (22.0)</td>
<td>26-42</td>
</tr>
<tr>
<td>Total</td>
<td>150 (100)</td>
<td>17-45</td>
</tr>
</tbody>
</table>
The MIC method cannot be used when atypical, amastigote (lacking flagella) *T. vaginalis* are present. Instead, a common *in vitro* lysis method for determining the sensitivity to antiprotozoal medications was used. Lysis of all trichomonas is an indicator of the efficacy of the test substance. Nutrient media containing trichomonas without metronidazole was used as a control. Metronidazole, at concentrations of 10, 15, 25, and 50 μg/ml was used in this experiment. Strains that lysed at a concentration of 25 μg/ml were considered resistant to metronidazole.

To determine sensitivity of clinical strains of *Trichomonas vaginalis* to CGNC, a method of cultivation on the medium for inoculation of trichomonas (MIT) was used. CGNC was added to the medium in a form of water solution with the final concentrations of 100, 200, 300, and 500 mg/ml. The control sample contained only suspension of trichomonad culture in nutritive medium.

The frequency of trichomonad detected in patients is shown in Table 2.

### Table 2

<table>
<thead>
<tr>
<th>Nosological form</th>
<th>Total examined</th>
<th>Number of strains detected</th>
<th>Frequency of detection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute diseases of urogenital tract (female)</td>
<td>9</td>
<td>2</td>
<td>22.2</td>
</tr>
<tr>
<td>Acute diseases of urogenital tract (male)</td>
<td>10</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>Chronic diseases of urogenital tract (female)</td>
<td>32</td>
<td>5</td>
<td>15.6</td>
</tr>
<tr>
<td>Chronic diseases of urogenital tract (male)</td>
<td>37</td>
<td>9</td>
<td>24.3</td>
</tr>
<tr>
<td>Sterility (female)</td>
<td>29</td>
<td>4</td>
<td>13.7</td>
</tr>
<tr>
<td>Sterility (male)</td>
<td>33</td>
<td>8</td>
<td>24.2</td>
</tr>
<tr>
<td>Total</td>
<td>150</td>
<td>30</td>
<td>20</td>
</tr>
</tbody>
</table>

Of the 30 isolates of *Trichomonas vaginalis*, 3 actively motile and 7 amastigote were selected for further investigation.

Trichomonas, at a concentration of ≥10^4 cells/ml were added to nutrient media. The ability of the protozoa to replicate in artificial nutrient media was used as a selection criterion. Characteristics of the trichomonas isolates and a description of clinical and anamnestic data of the patients are presented in Appendix.

Analysis of the sensitivity of various isolated strains of *T. vaginalis* to CGNC and metronidazole was conducted. The data from this experiment is shown in Table 4. Ten isolated strains of trichomonas were selected based on the selection criteria such as sufficient amount (≥10^4 cells/ml) and ability to multiply on the MIT were placed in test tubes with known amount of metronidazole or CGNC as per the method described in the section "Novelty of invention". Each test was repeated 3 times. Each isolate had a control such as medium for inoculation of trichomonas only - without any test-substance.

### Table 4

<table>
<thead>
<tr>
<th>Isolate number</th>
<th>Control (number of trichomonads, cells/ml)</th>
<th>CGNC (number of trichomonas, cells/ml)</th>
<th>Metronidazole (MIC, μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>actively mobile 5x10^4 cells/ml</td>
<td>weakly mobile 10^2</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>actively mobile 3x10^4 cells/ml</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>actively mobile 10^4 cells/ml</td>
<td>weakly mobile &lt; 10^2</td>
<td>25</td>
</tr>
</tbody>
</table>
Table 4 shows that all actively mobile strains were detected in case of primary infection. Patients with the amastigote form of *T. vaginalis* had a history of infection and were treated with antiprotozoal medication.

0 - no trichomonas present

Data in Table 4 show that strains 4, 6, 9, and 10 were resistant to metronidazole (MIC was greater than 25 μg/ml).

Isolated strains 2, 6 and 8 were lysed at a CGNC concentration of 100 mg/ml (Table 4). It has also significantly reduced the number of protozoa in the rest of the samples in comparison to the control.

A CGNC concentration of 200 mg/ml inhibited all growth of isolates 1-6 and 8. The number of trichomonas isolates 7, 9 and 10 were significantly less than in the control sample (Table 4).

A concentration of 300 mg/ml CGNC inhibited all growth of isolates 1-9 (Table 4). Isolate no.10 contained only <10 cells/ml of *T. vaginalis*.

Addition of 500 mg/ml CGNC to nutrient media led to the death of protozoa in all 10 samples (Table 4). Isolate no.10 contained only <10 cells/ml of *T. vaginalis*.

An increase in the concentration of CGNC in the growth medium correspondingly reduced the amount of nutritive substances necessary for trichomonads. In the control samples of isolates 4, 5, 8 and 9 there was abundant growth of associated bacterial flora. There was no growth of microbial flora in samples of the same isolates containing CGNC at ≥300 mg/ml. The results obtained revealed anti-trichomonad activity of CGNC at concentration of 200 mg/ml. A correlation among the type of *T. vaginalis*, previous courses of treatment, or the effect of metronidazole was not found. The antibacterial effect of CGNC on the accompanying flora was noted in patients with urogenital tract dysbacteriosis that had developed after previous use of antibiotic therapy. The effective MIC for 4 test samples was ≥300 mg/ml. Clinical and literature data show that diseases caused by *T. vaginalis* are chronic, are poorly treated with currently available therapeutic substances (metronidazole and derivatives of 5-nitrimidazole, tinidazole, nirmorazole, ornidazole, secnidazol, and derivatives of 4-aminquinoline such as chloroquine and nitrofuran). The use of antibiotics with a wide spectrum of activity in diseases of the urogenital tract can lead to microbiocenosis of the urogenital tract. This condition reduces the effectiveness of local non-specific immunity and is the main reason for complaints on itchiness, burning, irritation and dis-

<table>
<thead>
<tr>
<th>Isolate number</th>
<th>Control (number of trichomonads, cells/ml)</th>
<th>CGNC (number of trichomonas, cells/ml)</th>
<th>Metronidazole, (MIC, μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>100 mg/ml</td>
<td>200 mg/ml</td>
</tr>
<tr>
<td>4</td>
<td>amastigote 10⁴ cells/ml</td>
<td>amastigote &lt;10²</td>
<td>amastigote &lt;10²</td>
</tr>
<tr>
<td>5</td>
<td>amastigote 10⁴ cells/ml</td>
<td>amastigote &lt;10²</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>amastigote 3x10⁴ cells/ml</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>amastigote 10⁴ cells/ml</td>
<td>amastigote &lt;10²</td>
<td>amastigote &lt;10²</td>
</tr>
<tr>
<td>8</td>
<td>amastigote 5x10⁴ cells/ml</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>amastigote 10⁴ cells/ml</td>
<td>amastigote 10³</td>
<td>amastigote &lt;10²</td>
</tr>
<tr>
<td>10</td>
<td>amastigote 10⁴ cells/ml</td>
<td>amastigote 5×10²</td>
<td>amastigote &lt;10²</td>
</tr>
</tbody>
</table>

Legend
charges with an unpleasant odour. It has been established that these conditions cannot be easily treated. The bactericidal effect of the test substance, CGNC, can prevent dysbacteriosis.

[0044] The study demonstrated the potential use of CGNC as a treatment for various trichomonias infections.

1. CGNC has antiprotozoal activity against ten T. vaginalis isolates obtained from patients with acute and chronic diseases of the urogenital tract.

2. The test substance demonstrated a trichomonacidal and trichomonastatic effect. The organism was killed in 30% of cases at a concentration of 100 mg/ml CGNC. There was an inhibitory effect on growth at this concentration in 70% of cases. The growth of T. vaginalis was inhibited in 90% of cases at a concentration of 300 mg/ml.

3. CGNC killed actively motile trichomonas and trichomonas without flagella.

4. A CGNC-related antibacterial effect on the associated flora was noted in patients with dysbacteriosis that had developed after the use of antibiotic therapy. Therefore, CGNC is effective as a therapeutic substance for the treatment of diseases associated with trichomoniasis.

Examples of the preferred execution of invention

[0045] Below are examples of emulsion based on the therapeutic substance

Example 1.

[0046]

CGNC - 10.0 g

Double distilled water - made up to 100 g.

Example 2.

[0047]

CGNC - 30.0 g

Double distilled water - made up to 100 g.

Example 3.

[0048]

CGNC - 50.0 g

Double distilled water - made up to 100 g.

[0049] To prepare an emulsion (Examples 1-3), a mixing apparatus is loaded with calculated amount of CGNC, the paste is heated to 50°C, and calculated amount of double distilled water is added. Heating is stopped when the emulsion becomes homogeneous and the mixture is packed in glass bottles.

Demographic and clinical data on patients and characteristics of T. vaginalis strains included in study of anti-trichomonad activity of CGNC in vitro

<table>
<thead>
<tr>
<th>No. of strain</th>
<th>Sex</th>
<th>Age</th>
<th>Diagnosis</th>
<th>Data of anamnesis</th>
<th>Characteristics of strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>19</td>
<td>Acute vulvovaginitis</td>
<td>Patien B., first visit to doctor, first instance of disease, never had treatment</td>
<td>Actively mobile 5×10^4 cells/ml</td>
</tr>
<tr>
<td>No. of strain</td>
<td>Sex</td>
<td>Age</td>
<td>Diagnosis</td>
<td>Data of anamnesis</td>
<td>Characteristics of strain</td>
</tr>
<tr>
<td>--------------</td>
<td>-----</td>
<td>------</td>
<td>------------------------------------------</td>
<td>----------------------------------------------------------------------------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>20</td>
<td>Acute colpitis</td>
<td>Patient K., first visit to doctor, first instance of disease, never had treatment</td>
<td>Actively mobile 3 x 10^4 cells/ml</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>17</td>
<td>Acute urethritis</td>
<td>Patient P., first instance of disease, never had treatment</td>
<td>Actively mobile 10^4 cells/ml</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>24</td>
<td>Chronic colpitis</td>
<td>Patient S., multiple visits to doctor. Has disease for 2 years, T. vaginalis detected for the first time. Metronidazole preventive treatment was used after the first visit to doctor; after that, symptoms of acute inflammation were removed. Reoccurrence after 5 months. Anamnesis contains 4 courses of antibiotic treatment without positive changes.</td>
<td>Amastigote 10^4 cells/ml</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>41</td>
<td>Chronic colpitis, chronic adnexitis</td>
<td>Patient K., multiple visits to doctor. Acute trichomoniasis 7 years ago. Had treatment with various medications, including metronidazole.</td>
<td>Amastigote 10^4 cells/ml</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>28</td>
<td>Chronic colpitis, secondary sterility</td>
<td>Patient E., first visit to doctor (doesn’t consider herself as a patient). Was examined in relation to sterility and being in contact with other patients. T. vaginalis was detected for the first time. Her sexual partner has chronic trichomoniasis diagnosed 2.5 years ago. Had a course of preventive treatment with metronidazole.</td>
<td>Amastigote 3 x 10^4 cells/ml</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>22</td>
<td>Chronic prostatitis</td>
<td>Patient M., first examination. After a casual sexual contact had characteristic discharges, did not go to doctor. Self-treated with metronidazole (as per instruction for use), that led to disappearance of acute symptoms. But, 2 months later, had periodic low abdominal pains. Considers himself to be sick for 8 months.</td>
<td>Amastigote 10^4 cells/ml</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>38</td>
<td>Chronic prostatitis</td>
<td>Patient P., multiple visits to doctor. Was diagnosed with acute trichomoniasis 14 years ago. Had a treatment in dermo-venereal clinic. First reoccurrence 8 months after the treatment. Had another course of anti-protozoal therapy (doesn’t remember the name of medications). After the second course he had no subjective improvements that forced him to do self-treatment with analgesics during deterioration periods. Permanent stretching pains for the last 6 months pushed him to visit an urologist.</td>
<td>Amastigote 5 x 10^4 cells/ml</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>31</td>
<td>Chronic prostatitis, sterility, expressed oligospermia, astenospermia</td>
<td>Patient I., first examination, pathogen is detected for the first time. Diagnosed with prostatitis 5 years ago. Twice had treatment against Chlamydia and ureaplasmic infections, which reoccurred 8-10 months after antibiotic therapy. Metronidazole was included in treatment of clamidiosis.</td>
<td>Amastigote 10^4 cells/ml</td>
</tr>
</tbody>
</table>
Industrial applications

[0050] The results obtained from the study showed the potential use of CGNC as treatment for acute, chronic and asymptomatic trichomonas infections. The use of CGNC as antiprotozoal therapy allows for the acceleration of the treatment process, inhibition of *T. vaginalis* and associated pathogenic microflora in urogenital tract infections such as those found in dysbacteriosis.

Claims

1. Conifer green needle complex for use in the treatment of acute, chronic or asymptomatic Trichomoniasis infections of the urogenital tract.

2. Conifer green needle complex for use as claimed in claim 1, wherein the conifer green needle complex is at a concentration of at least 100 mg/ml and has trichomonadocitic or trichomonadostatic effects.

3. Conifer green needle complex for use as claimed in claim 1, wherein the conifer green needle complex is at a concentration of at least 300 mg/ml and suppresses the growth of *T. vaginalis*.

4. Conifer green needle complex for use as claimed in claim 1, wherein the conifer green needle complex is at a concentration of at least 500 mg/ml and kills *T. vaginalis*.

5. The use of conifer green needle complex for manufacture of a medicament for treatment of an infection chosen from the group comprising acute, chronic or asymptomatic Trichomoniasis infection.

6. The use of conifer green needle complex as claimed in claim 5, wherein the concentration of conifer green needle complex in the medicament is at least 100 mg/ml.

Patentansprüche


2. Koniferen-Grünnadelkomplex zur Verwendung nach Anspruch 1, worin der Koniferen-Grünnadelkomplex in einer Konzentration von zumindest 100 mg/ml vorliegt und trichomonadocitic oder trichomonadostatische Wirkungen aufweist.

3. Koniferen-Grünnadelkomplex zur Verwendung nach Anspruch 1, worin der Koniferen-Grünnadelkomplex in einer Konzentration von zumindest 300 mg/ml vorliegt und das Wachstum von *T. vaginalis* unterdrückt.

4. Koniferen-Grünnadelkomplex zur Verwendung nach Anspruch 1, worin der Koniferen-Grünnadelkomplex in einer Konzentration von zumindest 500 mg/ml vorliegt und T. vaginalis abtötet.

5. Verwendung des Koniferen-Grünnadelkomplexes zur Herstellung eines Medikaments zur Behandlung einer Infek-
Revendications

1. Complexe d’aiguilles vertes de conifère pour utilisation dans le traitement des infections aiguës, chroniques ou asymptomatiques par Trichomoniasis du tractus urogénital.

2. Complexe d’aiguilles vertes de conifère pour utilisation selon la revendication 1, le complexe d’aiguilles vertes de conifère étant à une concentration d’au moins 100 mg/ml et ayant des effets trichomonadocides et trichomonadosstatiques.

3. Complexe d’aiguilles vertes de conifère pour utilisation selon la revendication 1, le complexe d’aiguilles vertes de conifère étant à une concentration d’au moins 300 mg/ml et supprimant la croissance de T. vaginalis.

4. Complexe d’aiguilles vertes de conifère pour utilisation selon la revendication 1, le complexe d’aiguilles vertes de conifère étant à une concentration d’au moins 500 mg/ml et tuant T. vaginalis.

5. Utilisation du complexe d’aiguilles vertes de conifère pour la production d’un médicament pour le traitement d’une infection sélectionnée du groupe comprenant l’infection aiguë, chronique et asymptomatique par Trichomoniasis.

6. Utilisation du complexe d’aiguilles vertes de conifère selon la revendication 5, la concentration du complexe d’aiguilles vertes de conifère dans le médicament étant au moins 100 mg/ml.
REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader’s convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- RU 2320319 [0005]

Non-patent literature cited in the description

- GRODSTEIN F. Relation of tubal infertility to a history of sexually transmitted diseases [0002]
- TIKHOMIROV A.L. Urogenital trichomoniasis [0002]
- VIDAL. Therapeutic substances In Russia. 2000 [0008]
- NARCISI E.M. In vitro effect of tinidazole and furazolidone on metronidazole-resistant Trichomonas vaginalis [0009] [0012]
- ZAKHARKIV YU.F. Dependence of efficacy of etiootropic therapy of patients with trichomoniasis on sensitivity of strains of a pathogen to antiprotozoic drugs [0009]
- THOMASON J. L. Trichomonas vaginalis [0010]
- DEBBIA E.A. In vitro activity of metronidazole alone and in combination with clotrimazole against clinical isolates of Trichomonas vaginalis [0011]
- HONIGBERG B.M. Structure of Trichomonas vaginalis Donne [0011]
- BORCHARDT K.A. A comparison of the sensitivity of the In Pouch TV, Diamond’s and Trichosel media for detection of Trichomonas vaginalis [0011]
- NARCISI E.M. In vitro effect of tinidazole and furazolidone on metronidazole-resistant Trichomonas vaginalis [0013]