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تحديد عدد من الجوانب الفسيولوجية والكيميائية والنسيجية لتأثير خميرة Candida glabrata على الجهاز التناسلي للإناث من فئران المختبر البيضاء وعلاجها بمستخلص الروزماري المائي والأمفوتريسين-ب.

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المستخلص:
هدفت الدراسة الحالية إلى تحديد التأثيرات الفسيولوجية والبيوكيميائية والنسيجية لفطر Candida glabrata على الجهاز التناسلي الأنثوي للفئران، والدور العلاجي لمستخلص إكليل الجبل المائي في الدم والجهاز التناسلي طوال فترة التجربة، ومقارنة هذه التأثيرات بدور هذا المستخلص عند التآزر مع الأمفوتريسين-ب. استمرت التجربة لمدة (٢٨) يومًا. تم تقسيم (٣٥) فأرًا على ٧ مجموعات تحتوي كل مجموعة على ٥ فئران ، تبلغ أعمارهم (١٦) أسبوعًا ، والتي تم علاجها أولًا بهرمون الإستريدول عدا مجموعة السيطرة للحصول على الطمث الكاذب، حيث تم حقنها بـ ٠,٥ ملغ تحت الجلد لمدة ٦ أيام بمعدل حقنة كل يومين قبل بدء التجربة. وبعدها حقن جزء من هذه الحيوانات مهبليًا بفطر كانديدا جلابراتا كل يومين ولمدة ٦ أيام . بعد ذلك أُجريت التجربة .

الكلمات المفتاحية: اكليل الجبل المائي، امفوتيريسين-ب، كانديدا كلابراتا.

Determination of several physiological, chemical, and histological aspects of the effect of Candida glabrata on the female reproductive system of white mice and their treatment with rosemary aqueous extract and amphotericin-B.

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Abstract

The present study aimed to determine the physiological, biochemical, and histological effects of Candida glabrata on the female reproductive system of mice and the therapeutic role of rosemary aqueous extract in the blood and reproductive system throughout the experiment period and to compare these effects with the role of this extract in synergy with amphotericin-B. The experiment lasted for (28) days. (35) mice were divided into 7 groups, each containing 5 mice, aged (16) weeks, which were first treated with estradiol hormone, except for the control group, to induce false menstruation, as it was injected with 0.5 mg subcutaneously for 6 days at a rate of one injection every two days before the start of the experiment. After that, a portion of these animals was vaginally injected with Candida glabrata fungus every two days for 6 days. After that, the experiment was conducted.

Keywords: rosemary aqueous , amphotericin-B , *Candida glabrata*

Introduction

Candida species belong to the oral, gastrointestinal, and vaginal microbiota and are responsible for a wide range of clinical manifestations, ranging from mucosal and skin overgrowth to bloodstream infections. C. glabrata was once believed to be nonpathogenic; it has quickly been linked to several illnesses in humans. These conditions are becoming more common and challenging to treat, particularly in individuals who have immunosuppression of any kind. These challenges result from C. glabrata's great resistance to traditional antifungal treatments and its capacity to create biofilms. Currently, C. glabrata is the second or third most common cause of systemic or superficial (oral, esophageal, vaginal, or urinary) Candida infections, which are frequently acquired in hospitals. However, little is currently understood about the body's defense systems that prevent infection, as well as the known virulence factors of C. glabrata. Azoles may be used to treat C. glabrata infections, although amphotericin B is frequently needed to eradicate this kind of fungus. Because of its medicinal qualities, rosemary extract has also been utilized in traditional medicine. According to some, rosemary helps strengthen the immune system, lessen muscle soreness, and enhance circulation and memory. It might, however, interfere with some drugs.

The Mediterranean region is home to the fragrant, evergreen herb rosemary. In addition to its possible health benefits, it is used as a culinary spice and an ingredient in perfumes. Rosemary belongs to the mint family, along with oregano, thyme, lavender, and basil. It is usually prepared as a powdered extract, a whole dried herb, or a tea made

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from either fresh or dried leaves. Since ancient times, the herb has been valued for its therapeutic qualities. The purpose of this study was to determine whether rosemary (Rosmarinus officinalis) leaf powder, either by itself or in conjunction with amphotericin B, a polyene chemical frequently used to treat systemic fungal infections, might eradicate fungi that infect the female reproductive tract. Amphotericin B binds to ergosterol, the primary constituent of the fungus cytoplasmic membrane, just like other polyene antifungals. When it is present in the right amounts, it perforates this membrane, allowing potassium to escape the cell and ultimately causing fungal cell death.

Consistency

Fallopian Tube

The fallopian tube is one of two tubes located on either side of the uterus, connecting it to the ovaries. The egg travels from the ovaries to the uterus through these tubes. They contain cilia that aid their passage. If inflammation occurs in this tube, it narrows or even closes, preventing the egg from reaching the uterus. Its wide end starts at the ovary. Its narrow end opens into the uterus from its upper side. The fallopian tube has a length of about 12 cm. Sperm fertilizes the egg in this tube. A layer of mucous membrane with many folds and papillae—small, cone-shaped projections of tissue—lines the fallopian tube. Three layers of muscle tissue cover the mucous membrane: an outside layer with longitudinal fibers that terminate in finger-like branches, a middle layer with circular fibers, and an inner layer with spirally arranged fibers. The infundibulum is the term for the chorionic villi that are situated close to the ovaries. The wide, distal (outside) portion of each fallopian tube is called the infundibulum, and it is responsible for capturing and moving the released eggs.

To direct the released eggs during ovulation, the tips of the villi protrude above the ovary and constrict close to the ovarian surface. The ampulla is the lengthy, middle portion of the fallopian tube that extends from the infundibulum. The isthmus, which joins the uterus to the ampulla and infundibulum, is a little region that is roughly 2 cm long. The fundus of the tube, the last portion of the fallopian tube, is found at the top. The uterine cavity, where the fertilized egg often attaches and develops, is reached by the fallopian tube, a thin canal that is attached to the isthmus and passes through the thick uterine wall. The narrowest portion of the tube is called the fallopian tube. Sperm and eggs are transported and kept viable by secretions secreted by the mucous membrane lining the fallopian tube. Calcium, sodium, chloride, glucose (a kind of sugar), proteins, bicarbonate, and lactic acid are the primary constituents of this fluid. Sperm need lactic acid and bicarbonate to use oxygen and support the growth of the egg following fertilization. The remaining chemicals create an environment that is conducive to fertilization, while glucose supplies nutrition for the sperm and egg. A woman's fertility

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may be impacted by fallopian tube abnormalities or injury. Implantation might not be possible, for instance, if the tubes are damaged or obstructed, if sperm cannot reach the egg due to a yeast infection, or if the fertilized egg cannot enter the uterus.

Candida glabrata

The number of Candida species infections has been rising over the past few decades. These infections can be systemic or local, and they have a high morbidity and fatality rate. Candida glabrata is one of the most common pathogenic fungi in humans, second only to Candida albicans. In addition to its strong resistance to antifungal medications and inability to produce hyphae or secret hydrolases, C. glabrata retains many virulence characteristics that contribute to its extreme aggressiveness, low treatment response, and significant recurrent candidiasis, such as the ability to form biofilms. Biofilms are highly resistant to antifungal treatments due to their remarkable organization, particularly concerning the intricate structure of the matrix. As a result, novel methods for treating the biofilms of C. glabrata are being developed. Because they lacked hyphae, C. glabrata strains were historically categorized under the genus Torulopsis. However, it was shown in 1978 that the ability to create hyphae or pseudohyphae was not a reliable way to distinguish between different species of Candida, and because of its propensity to cause illness in humans, C. glabrata was suggested for classification within the genus Candida. Compared to Candida albicans (4–6 µm), Candida tropicalis (4–8 µm), and other species, the cells of Candida glabrata (1–4 µm) are substantially smaller. On Sabouraud dextrose agar, C. glabrata strains form glossy, smooth, cream-colored colonies that are nearly identical to those of other Candida species, despite their comparatively tiny size (Urbieta et al., 88).

Virulence Factors and Pathogenicity

Millions of individuals worldwide suffer from fungal infections, with an estimated 1,350,000 fatalities annually. Candida species are among the pathogenic fungi that cause candidiasis, the most prevalent invasive fungal illness in affluent nations. As members of the natural microbiota of the oral cavity, gastrointestinal system, and vaginal tract, Candida species coexist as commensals on mucosal surfaces. However, under certain circumstances, such as host-disrupted microbiota or immunocompromised hosts, they might opportunistically become pathogenic and cause clinical symptoms ranging from bloodstream infections to mucocutaneous overgrowth. In addition to making up 60% of all Candida species found in the human body, Candida albicans and Candida glabrata are

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the most common pathogenic species, causing over 400,000 potentially fatal infections annually throughout the world.

The expression of fungal virulence factors and the host immune system interact intricately in the contact between Candida and its host cells, and the presence of additional microbes influences this interaction. (Galocha, et al., 2019).

It should be noted that the increasing use of azole antifungals for the treatment of Candida glabrata superficial and systemic infections has led to the selection and development of resistance isolates, as well as an increase in infections by other non-albicans species. Moreover, it is believed that C. glabrata sensu stricto is the most pathogenic species, followed by C. nivariensis and C. bracarensis.

The pathogenicity of the various Candida species is influenced by virulence factors, particularly in patients with impaired immune systems. C. glabrata can attach itself to invasive medical equipment and host cells by secreting hydrolytic and proteolytic enzymes. Similarly, it has a large amount of beneficial genomic flexibility and can create biofilms that offer antifungal resistance. As a result, it is regarded as an aggressive yeast for people.

There is little information available regarding the different virulence factors that the C. glabrata complex species employ. However, given the increasing number of illnesses these fungi cause and their high incidence of antifungal resistance, it is crucial to understand the virulence features displayed by the C. glabrata complex (De-León et al., 2021).

The production of hydrolytic enzymes (such as phospholipases, proteases, and hemolysins), sticking to susceptible host surfaces, eluding host defenses, and replicative aging are the virulence factors associated with pathogenicity in C. glabrata. These factors enhance the initiation of infection. Other pathogenic factors include iron regulation and genetic alterations. Thus, biofilm formation, resistance to neutrophil mortality, tolerance to high-stress environments, and the development of resistance to antifungal drugs, especially fluconazole and other azole derivatives, were recorded. (Galocha et al., 2019). Among the mechanisms found are the overexpression of drug transporters, gene mutations that alter thermotolerance, the emergence of hypervirulence due to increased adhesion factors, and modifications in critical enzymes that produce cell wall proteins that obstruct the action of drugs meant to inhibit them. The C. glabrata complex was also found to possess virulence factors, including the production of hemolysins, phospholipases, and proteases, as well as the formation of biofilms that allow the complex to develop fungal resistance and evade the host immune response. Consequently, the pathogenetic combination of the C. glabrata complex is perfect for infiltrating the immunocompromised host.

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MATERIALS AND METHODS

The experiment was designed using 35 white female rats distributed in 7 cages. The first cage was a negative control, and the second was a positive control, injected with Candida glabrata fungi, which were obtained from infected women, and this study focused on injecting these fungi into the vagina of white female rats. The injection process was carried out in a way that ensures that the vagina is infected by creating favorable conditions and atmospheres capable of vaginal infection through the injection of the hormone (estradiol benzoate). When estrogen is the dominant sex hormone, the uterus is vulnerable to infection with fungi that can be detrimental to survival and fertility. Some results indicate that sex hormones influence the existence of broad-spectrum bactericidal agents in the uterine secretions of mice. Further studies indicate that the epithelial cells lining the uterine cavity are a major source of antifungal activity, and mucus-secreting glands border the passage through the cervix. Except right before ovulation. The mucus's consistency changes during ovulation, allowing sperm to pass through it for fertilization to take place. Consequently, ovulation and fake menstruation are brought on by estradiol injections.

Estradiol benzoate (0.5 mg subcutaneously) was injected every two days, and six days after the first dose of estradiol, the fungus was injected directly into the vagina. The percentage of Candida glabrata injections was ($6\times〖12〗^{4}$) in the injection fluid. Also, amphotericin-B was administered directly, as well as they were given in synergy with the aqueous extract of rosemary, according to the group, and this process of dosing was done orally, where the proportion of amphotericin-B administration was (20 mg/kg).

Experience design

The animals were distributed into 7 groups, and each group included 5 animals with close weights, as follows:

1- A healthy control group of rats (white females).
2- A positive control group of females infected with the fungus (Candida) **C**
3- A group of animals treated with aqueous extract (of rosemary) **R**
4- A group of animals treated with (amphotericin-B) **A**
5- A group of animals infected with the fungus (Candida and aqueous extract) **C+R**
6- A group of animals infected with the fungus (Candida and amphotericin-B) **C+A**
7- A group of infected animals (Candida, amphotericin-B, and aqueous extract). **C+A+R**

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Result and discussion:

Standards	before the experiment		After the experiment is over	
the group	IGM **(mIU/ml)**	IGG **(mIU/ml)**	IGM (mIU/ml)	IGG **(mIU/ml)**
control	25.02±0.4 A b	320.50 ±0.5 A c	25.30±0.1 B b	310.01 ±1.1 B c
injured with fungi **C**	25.00±0.1 B b	322.50 ±0.5 B c	44.00±0.5 A a	390.60 ±0.0 A a
treatment with extract **R**	26.00±0.3 A a	325.00 ±1.0 A b	22.40±0.1 B d	310.21 ±0.1 B c
treatment in the property **A**	25.21±0.5 A b	324.00 ±1.0 A b	25.00±0.1 B b	321.04 ±0.3 B b
infected with **C** and treat **R**	25.05±1.0 A b	327.50 ±0.5 A a	24.50±0.2 B c	311.20± 0.5 B c
infected with **C** and treat	26.30±0.0 A a	336.00 ±1.5 A a	26.20±0.9 B b	325.48± 0.8

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A				B b
infected with **C** and treat **A + R**	25.50±0.2 A b	323.00 ±1.0 A c	24.31±0.1 B c	300.50 ±1.1 B d

-The values are mean ± standard error.
- Small letters indicate the significant changes per column at the level of significance ($P \leq 0.05$).
Capital letters indicate the significant changes for one grade at the level of significance ($P \leq 0.05$-

Result and discussion (1):

Immune system variables (IgG, IgM) were measured in female mice before and at the end of the experiment. There was no change in the immune system variables from the normal range before the experiment. Post-experimental immune system variables showed a significant increase ($P \leq 0.05$) in IGM and IGG levels in the fungal-infected group compared to the control group. Both the rosemary aqueous extract-treated and amphotericin B-treated mice showed normal IGM and IGG levels. Treatment of mice with rosemary aqueous extract resulted in a significant decrease in serum IgM and IgG levels compared to the control group. Furthermore, administration of amphotericin B to mice with rosemary aqueous extract, even in the Candida-infected group, resulted in a reduced infection rate.

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Table (2) Results of the concentrations of analyses in rat blood serum (CRP, CD4, SGOT)

Standards / the group	CRP (mg/L)	CD4 (cells/µl)	SGOT (U\L)
control	10.68±0.8 b	1190±0.0 B	45.91±0.8 a
injured with fungi **C**	18.43±0.1 a	710±0.1 D	19.65±0.3 d
treatment with extract **R**	10.36±0.7 b	1200±0.5 A	45.92±1.0 a
treatment in the property **A**	10.91±0.2 b	1184±0.2 B	44.17±0.2 c
infected with **C** and treat **R**	10.75±0.2 b	1150±0.1 C	44.72±0.2 b
infected with **C** and treat **A**	10.51±0.3 b	1155±0.0 C	45.08±1.1 b
infected with **C** and treat **A + R**	9.46±0.2 c	1210±0.0 A	46.50±0.2 a

Values are the mean ± standard error.

- Letters indicate significant changes for each column at the significance level ($P \leq 0.05$).

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Result and discussion (2) :

It is noted from Table (2) that treating mice with Candida fungi resulted in a significant increase in (CRP) C-reactive protein and liver enzymes (SGOT) (Serum Glutamic Oxaloacetic Transaminase) compared to the control group. Also, it led to a decrease in the number of lymphocytes (CD4) (Cluster of Differentiation 4) compared to the control group, indicating the presence of significant inflammation and a decrease in the immunity of laboratory animals due to infections caused by the fungi. However, treating mice with the aqueous extract of rosemary led to a significant decrease in the percentage of C-reactive protein and liver enzymes (SGOT) (Serum Glutamic Oxaloacetic Transaminase) and an increase in the number of lymphocytes (CD4) (Cluster of Differentiation 4) in the blood serum compared to the control group. Also, giving amphotericin B to mice alone and in synergy with the aqueous extract of rosemary, even in groups infected with Candida fungi, led to results that did not differ significantly compared to the healthy control group**.**

Images of fallopian tube tissues of female mice.

The fallopian tube tissues showed no serious or dramatic histological changes but were all normal in all groups, whether the control group, the group treated with rosemary aqueous extract, or the group treated with amphotericin B, except for the second group exposed to fungi, where the tissues showed the presence of clumps in the middle of the fallopian tube with blood clots and the absence or significant reduction of cilia.

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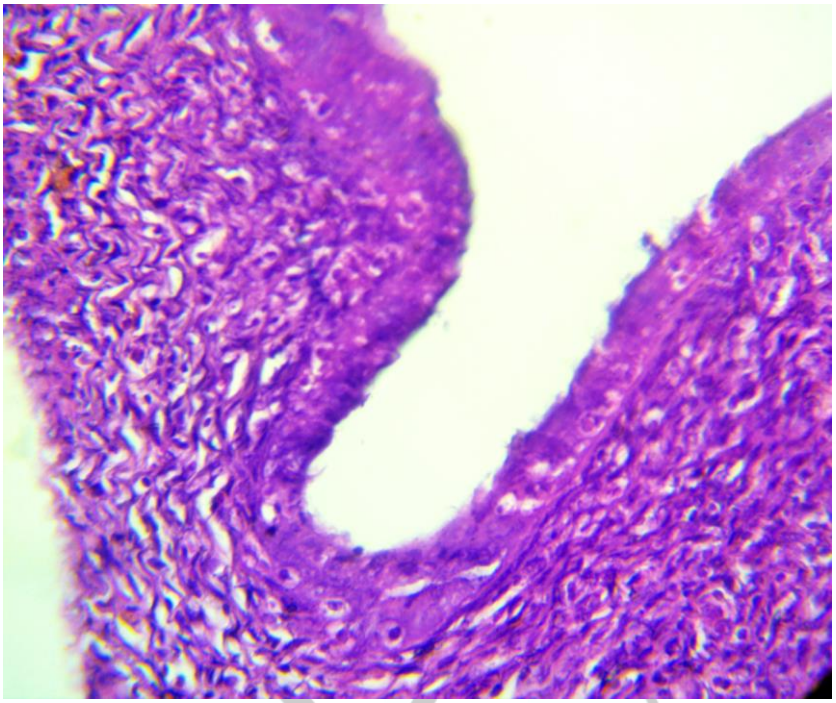

Figure (1) Cross-section of the fallopian tube in control group animals.

A: Fallopian tube cavity B: circular muscles C: ciliated cell D: peg cell F: lamina propria

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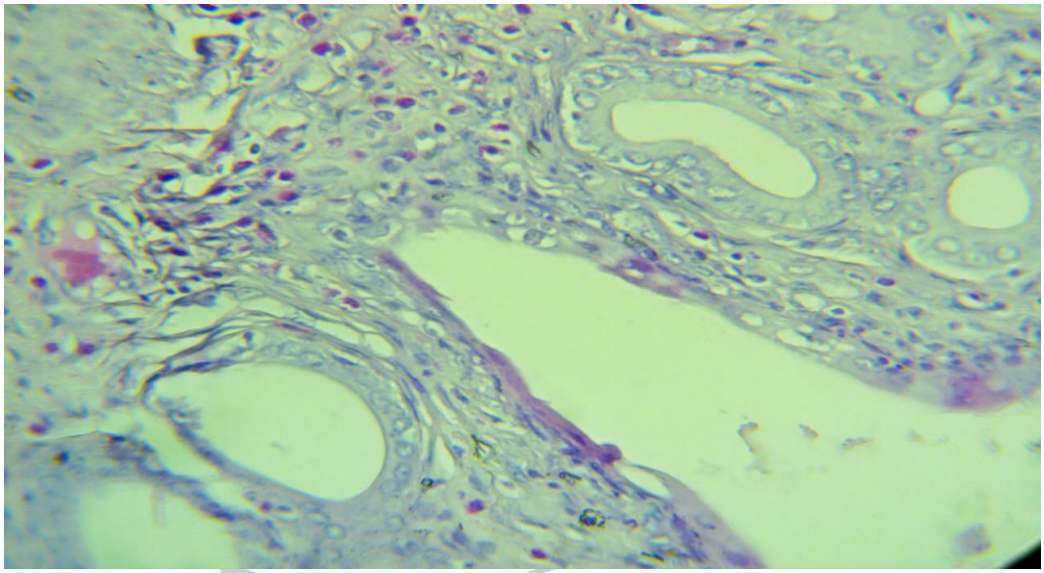

Image No. (2) A cross-section of the fallopian tube tissue of animals exposed to Candida fungi.

A: Fallopian tube cavity B: cellular debris C: blood clot

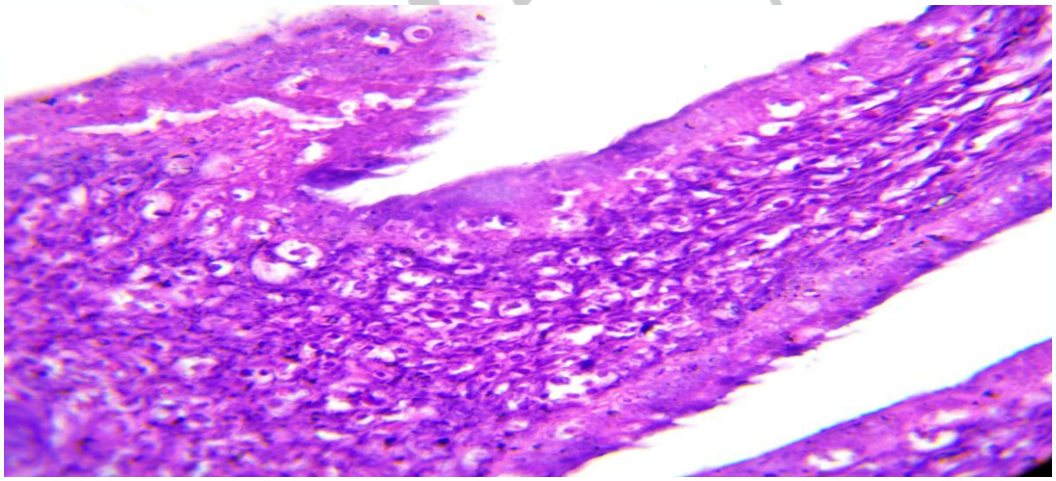

Image No. (3) A cross-section of fallopian tube tissue from animals treated with rosemary extract.

A: Fallopian tube cavity B: ciliated cell C: peg cell D: lamina propria

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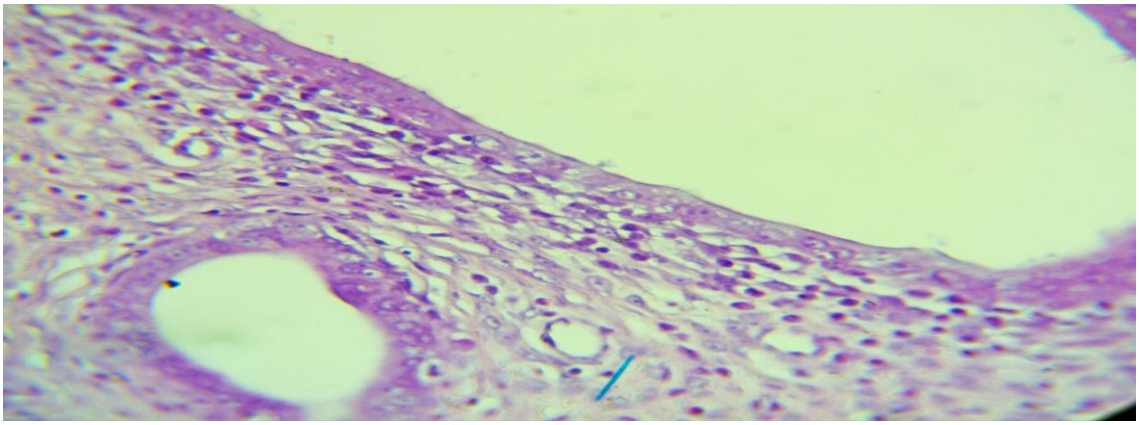

Picture No. (4) a cross-section of the fallopian tube tissue of animals dosed with amphotericin-B in synergy with aqueous extract of rosemary

A: Fallopian tube cavity B: ciliated cell C: lamina propria D: circular muscles

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