

DETERMINATION OF A NUMBER OF PHYSIOLOGICAL AND IMMUNOLOGICAL ASPECTS OF THE EFFECT OF *CANDIDA ALBICANS* ON FEMALE RATS AND THEIR TREATMENT WITH AQUEOUS EXTRACT OF DANDELION ROOT AND FLUCONAZOLE

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Abstract

The current study aimed to know the physiological, immunological and biochemical effects of *C. albicans* fungi on the female reproductive system of rats and the therapeutic role of the aqueous extract of dandelion roots in the blood and the reproductive system throughout the duration of the experiment, and to compare these effects with the role of this aqueous extract of dandelion roots in synergy with tetracycline and flucanazole.

The experiment started in May 20/04/2021 until 1/6/2021 for a period of (42) days. The number of (35) rats of (12-14) weeks old were distributed, and the animals were distributed into two groups, the first group being infected by (20) A rat was first treated with the hormone estradiol, where it was injected with 0.5 mg subcutaneously for 6 days every two days, and then a section of these animals was injected vaginally with *C. albicans* and also for 6 days, with an injection every two days, and then the experiment was conducted, as all the animals were distributed On 7 groups, each group has 5 rats.

Introduction

Candida is a prevailing symbiont type of fungus that occupies the oral cavity, pharynx, gastrointestinal tract, vagina, and the skin of healthy people that reaches 50% of total population. The natural balance of *Candida*, which leads to the transition from normal flora¹² to pathogenic and opportunistic infections.

Recently, *Candida* was detected in the lesions of root caries in the teeth and that *Candida* has an effect on the microbial environment and causing caries in the root of the tooth, as well as individuals who do not suffer from tooth decay found a significant increase in *C. albicans* fungi between the gums and the inner edges of the teeth. Other studies showed that *C. albicans* increased carcinogenesis in biofilms by altering their microbial environment, which led to the production of a multimicrobial biofilm with increased acidity, thus exacerbating the demineralization of teeth⁴. Finally, *C. albicans* is a common fungus symbiont with humans and is generally harmless in healthy people. Numerous variables can lead to the overgrowth of *C. albicans* and induce a range of complexities inside the host from local superficial infection to life-threatening spread *Candida*¹⁰.

In this study, the roots of dandelion were chosen for its great importance and origin. The use of this plant for healing purposes began before human history. Dandelion is famous conventional

herbal plant utilized in many medical fields to heal a number of ailments extending from wounds to diabetes. The liquid gathered from this plant is used as a traditional medicine for treating uterine cancer and tumors. This herb is famous for its roots, which are can be added to coffee, because it gives a bitter taste with zero caffeine. The roots of the plant are also reported to have 40% inulin as well as act as an anti-diabetic agent. Historically, the ancient Egyptians cultivated the plant as a medicinal plant. The medicinal properties of the dandelion plant include hepatoprotective, antidiabetic, anticancer, anti-inflammatory, analgesic, antioxidant, antimicrobial, antimalarial cardiovascular, anthelmintic, and anti-allergic activities³.

Note that fluconazole therapy has properties that make it heavily employed in the clinical treatment of *C. albicans* infection. *C. albicans* however possesses the ability to acquire fluconazole resistance. A promising approach to increasing the efficacy of fluconazole is to identify potential drug targets that are able to improve the antifungal influence of fluconazole⁸.

Consistence

uterus

The uterus was considered to be free of microorganisms. However, an uninfected bacterial community was detected in the uterus of humans and other mammals, and endometrial microbiota may be linked with uterine diseases whether benign or malignant. There are many evidences which indicate that Lactobacillus is the most ample genus in healthy endometriosis and generally it is evident that in pathological and infertile cases the existance of this genus is low⁹.

Some viruses may cause cervical cancer, which is the fourth most common female malignancy in females worldwide. Epidemiological reports have shown that cervical cancer is a global health problem among women in developing countries in particular, thus creating an economic and medical burden in the community¹.

Candida albicans

Candida is a prevalent symbiont type of fungus that occupies the oral cavity, pharynx, gastrointestinal tract, vagina, and skin of healthy people with 50% of total population. The diverse clinical signs of Candida species extend from localized cursory mucocutaneous disorders to maladies that include different organ systems and are life-threatening. Different variables lead to unsettling influences within the normal balance of Candida driving to a move from the ordinary flora to pathogenic and opportunistic infections. Infections develop because of the immune weakening of the infected person. The intestinal mucosal barrier is damaged. The symbiosis relationship is present as long host's immune system are somehow equal to the virulence components of Candida¹²

The cell wall of fungus can be identified outside the membrane of plasma and cell compartment mediates all of the cell's relationships with the environment. Also, it works to protect all what the cell contains and gives rigidity and determines the structure of the cell as well. Also, the cell wall is a strong structure that enjoys high flexibility which works on protecting the cell from various issues, including osmotic changes where the cell wall allows interaction with the external environment because some of its proteins are adhesives and receptors, some components of the wall can to drive the host's immune response⁵. The cell wall of candida contains an internal

skeletal layer of β -glucan, chitin and an external layer of glycosylated proteins. Such proteins perform key parts in host cell adhesion¹³. The fungal wall contains primarily glycoproteins. This wall is a great target for antifungal treatment, as components of the parasitic cell wall are do not exist in people⁵. In the past decades, *Candida* has acted as a major causative agent of life-threatening infections with death rates close to 40 per cent in spite of the treatment. This fungus is found in three biological stages: yeast, pseudohyphae, and hyphae. Hyphae, which represents an important stage in the disease process, can cause tissue damage. By invading mucosal epithelial cells and then leading to blood infection in addition, their transformation from yeast to hypha can help escape phagocytosis, increasing the potential for invading host tissues and causing greater damage².

Virulence Factors and Pathogenicity

Fungal infection is a major factor in infectious disease-related deaths worldwide, with *Candida albicans* predominate as the primary reason of invasive candidiasis. Considering that fungi are eukaryotic similar to their human host, quantity of unique molecular objectives which can be used for antifungal development is still at low level. Presently, we can find only specific types of drugs accepted for treating invasive onychomycosis and additional drug-resistant types of fungi have emerged, such as *Candida auris* and *Candida glabrata*⁷.

Candida albicans infection ranges from cursory to systemic and is one of the main causes of fungal-associated nosocomial infection, indicating that the host environment does a major part in modulating the host-pathogen interaction. Additionally, *C. albicans* can remodel its cell wall in response to the circumstances of the environment to avoid mechanisms of host clearance and infection establishment in the oral and vaginal mucosa, and macrophages play a major role in eliminating *C. albicans*.

The influence of fungal microbes on the health of human is becoming more clear. *Candida albicans* is a prevaalent symbiont fungus which leads to local and systemic infections, especially in immunocompromised patients and this may lead to death. However, *Candida* has also been significantly associated with a variety of inflammatory disorders, which have conventioanlly been viewed as episodic⁶. Besides mechanisms of drug resistance, *Candida* is highly adaptable, enabling transmission from symbiont to pathogen precisely because of a range of virulence factors, the ability to change shape and form biofilm is fundamental in the pathogenesis of *Candida albicans*. In fact, most infections with this pathogen are associated with biofilm formation on the surfaces of hosts or medical devices, causing significantly higher rates of disease and mortality, the biofilm formed by *Candida* is inherently of low sensitivity to treatment. Candidalysin secretes the human pathogen Candidalysin and is essential for mucosal and systemic infection and is a major driver of host cell activation, leukocyte (neutrophil) recruitment, and immunity. Candidalysin was discovered in 2010 and is considered essential for mucosal infection and is admitted to trigger epithelial cells to stimulate innate immune responses which are linked with protection or immune pathology during oral or vaginal infections. Besides, Candidalysin stimulates mononuclear macrophages¹¹.

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 Albicans* fungi, fungi 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 (Estradiole 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 the channel through the cervix is lined with glands that secrete mucus. Except just before ovulation occurs. At ovulation, the consistency of the mucus changes so that the sperm can swim through it and fertilization can occur at this time. Therefore, the injection of estradiol causes ovulation and false menstruation. 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 Albicans* injections was 3×10^4 in the injection fluid.. Also, Fluconazole was administered directly, as well as they were given in synergy with the aqueous extract of dandelion roots, according to the group, and this process of dosing was done orally, where the proportion of Fluconazole administration was (50 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 dandelion roots) **H-**
- 4- A group of animals treated with Fluconazole **F-**
- 5- A group of animals infected with the fungus (Candida and aqueous extract) **C+H-**
- 6- A group of animals infected with the fungus (Candida and fluconazole) **C+F-**
- 7- A group of infected animals (Candida, fluconazole and aqueous extract). **C+F+H-**

RESULT :

The proportion of immunological variables (IGG, IGM) for female rats was measured before the experiment began, a week after the dosing process and at the end of the experiment. One of the dosing process, where it appeared that all groups had a significant increase in the infection rate except for the control group and the group dosed with aqueous extract of *Cichorium intybus* and the group dosed with fluconazole, while the rest of the groups showed a significant increase in the rate of infection. Immunological tests were also conducted after the end of the experiment, and the results showed no change in the result of the control group and the group dosed with two fluconazole while the treatment of rats with *C.Albicans* fungi in the second group led to a significant increase ($P \leq 0.05$) in (IGG, IGM) compared with The control group, while the

treatment of rats with aqueous extract of Cichorium intybus led to a significant decrease in the proportion of (IGG, IGM) in the blood serum compared with the control group, as well as the administration of rats with fluconazole in synergy with the aqueous extract of dandelion roots, even in groups infected with Candida fungi . to a decrease in the incidence of infection

Table (1) C. albican fungi on some immunological variables in the serum of rats

Standards	before the experiment		after a week		After the experiment is over	
	IGM (mIU/ml)	IGG (mIU/ml)	IGM (mIU/ml)	IGG (mIU/ml)	IGM (mIU/ml)	IGG (mIU/ml)
control	20.00±0. 4 C c	221.50±0 .5 C c	20.16±0.0 A c	229.31± 0.2 A c	20.32±0. 1 B c	224.91±1 .1 B c
injured with fungi C	20.00±0. 1 C c	220.50±0 .5 C c	47.55±0. 6 A a	280.49±0. 2 A a	43.80±0. 4 B a	260.98±0 .2 B a
treatment with extract H	22.00±0. 4 C b	223.00±1 .0 C b	21.47±0. 4 A c	223.16±0. 3 A c	18.90±0. 4 B c	220.27±0 .1 B c
treatment in the property F	21.00±0. 4 C b	222.00±1 .0 C b	20.81±0. 2 A c	221.41±0. 4 A c	20.27±0. 1 B d	221.02±0 .3 B d
infected with C and treat H	21.00±1. 0 C a	223.50±0 .5 C ab	51.20±0. 4 A b	307.81±1. 1 A b	38.77±0.6 B b	288.40±0. 5 B a
infected with C and treat F	23.00±0. 0 C a	224.00±1 .5 C a	47.40±0. 9 A b	266.30±0. 1 A b	31.28±0. 9 B b	245.48±0. 8 B c
infected with C and treat F + H	23.50±0. 5 C a	223.00±1 .0 C ab	50.58±0. 9 A b	304.33±2. 4 A b	34.81±0. 6 B c	234.56±1 .1 B c

It is noted from Table (2) that the treatment of rats with *Candida* fungi led to a significant increase in the immunoassays for interleukin (10.6) compared with the control group, while the treatment of rats with aqueous extract of *Cichorium intybus* led to a significant decrease in the percentage of the immunoassays for interleukin (10, 6) in the blood serum compared with the control group, as well as the administration of fluconazole to rats alone, as well as in synergy with the aqueous extract of *Cichorium intybus*, even in groups infected with *Candida* fungi, led to results that are not significantly different compared to the healthy control group. As for interleukin (12), treatment of rats with *Candida* fungi led to a significant decrease in the immunological tests for interleukin (12) compared with the control group, while treatment of rats with aqueous extract of *Cichorium intybus* led to a significant increase in the percentage of immunological tests for interleukin (12) in serum blood compared with the control group.

Table (2) results of the concentrations of interleukins in the blood serum of rats (IL12, IL10, IL6).

-The values are mean \pm standard error.

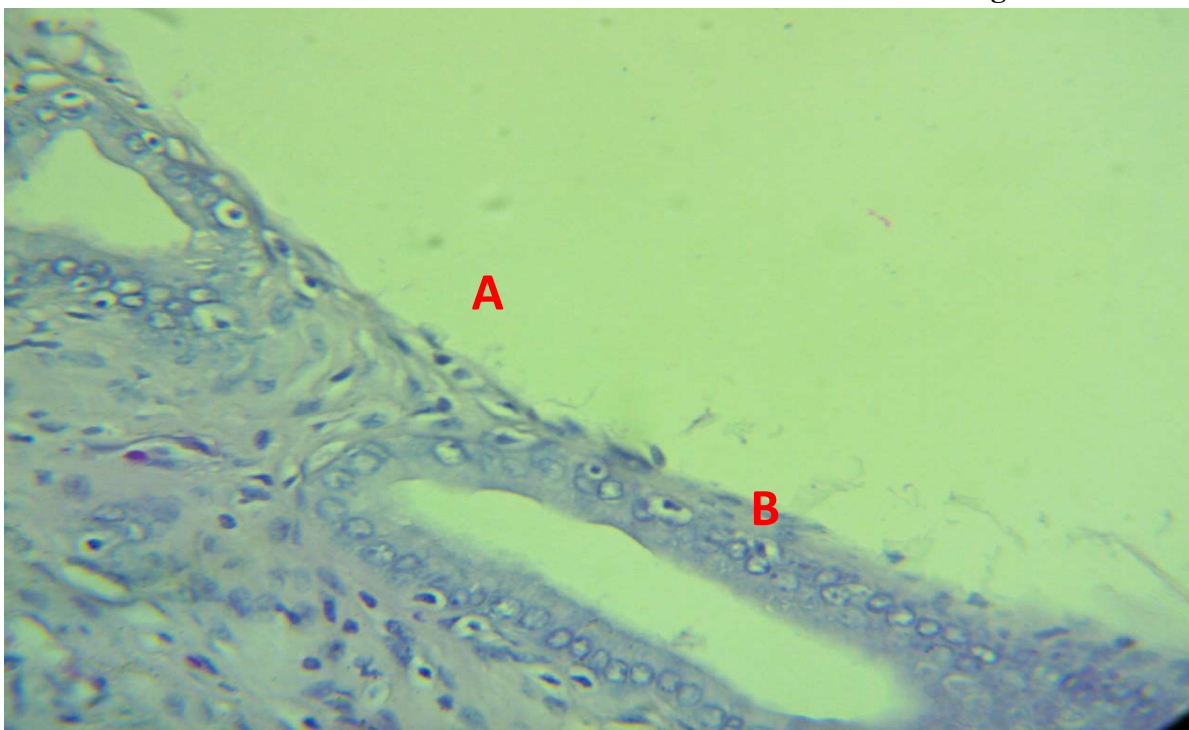
- Lower case letters refer to the important changes per column at the level of significance ($P \leq 0.05$).

) Capital letters indicate the significant changes for one grade at the level of significance ($P \leq 0.05$).

Standards the group	IL 12 (pg/ml)	IL 10 (pg/ml)	IL 6 (pg/ml)
control	145.68 \pm 0.8 a	6.64 \pm 0.0 c	19.91 \pm 0.8 c
injured with fungi C	95.43 \pm 0.1 c	10.89 \pm 0.1 a	28.65 \pm 0.3 a
treatment with extract H	150.36 \pm 0.7 a	5.29 \pm 0.5 c	16.92 \pm 1.0 c
treatment in the property F	141.91 \pm 0.2 a	6.85 \pm 0.2 c	21.67 \pm 0.2 c
infected with C and treat H	140.75 \pm 0.2 b	6.41 \pm 0.1 b	19.72 \pm 0.2 b
infected with C and treat F	140.51 \pm 0.3 b	6.97 \pm 0.0 b	21.38 \pm 1.1 b
infected with C	140.46 \pm 0.2	6.07 \pm 0.0	21.50 \pm 0.2

and treat F + H	b	b	b
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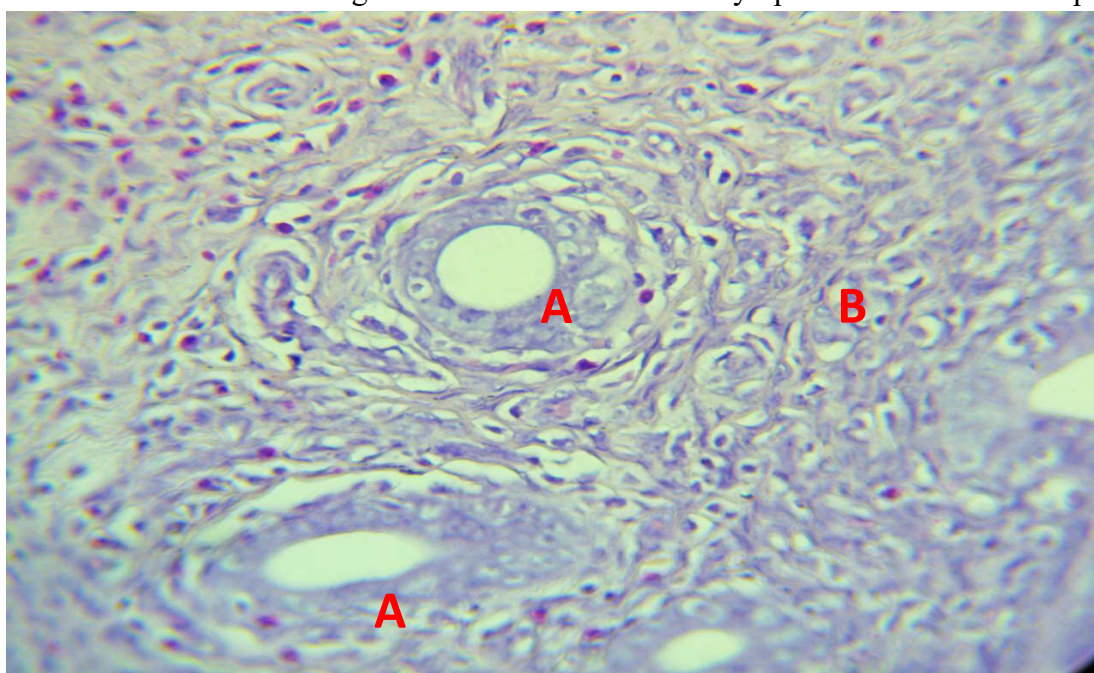
.Uterine tissue images of female rats



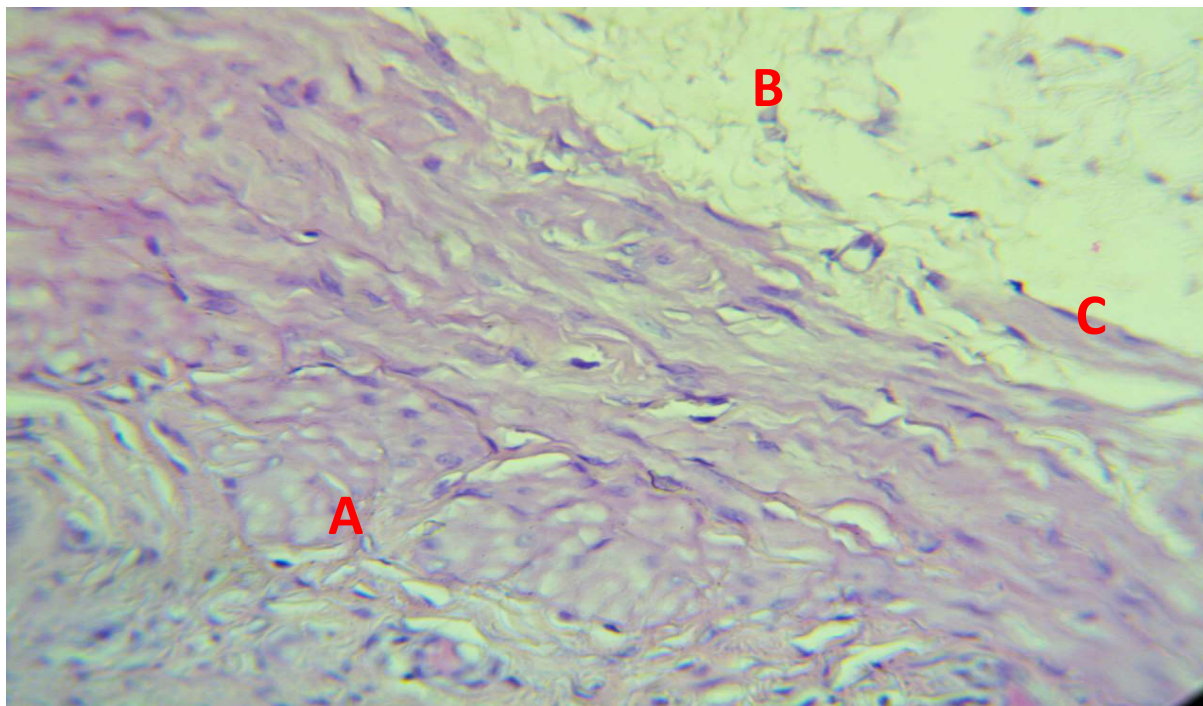
Picture No. (4-1) a cross-section of the uterine tissue of animals exposed to Candida fungi

A: The disappearance of the epithelium lining the uterine lining

B: The cells of the uterine glands in which some of the cytoplasm of these cells erupt



Picture No. (4-2) a cross-section of the uterine tissue of animals dosed with aqueous extract of
Cichorium intybus
A: uterine glands B: Detached ligament tissue with fibroblasts



Picture No. (4-3) a cross-section of the uterine tissue of animals dosed with fluconazole in
synergy with aqueous extract of Cichorium intybus
A: muscular layer of the uterus B: A soft connective tissue that represents the membrane around
the uterus
C: fibroblasts

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