OPEN ACCESS
Saudi Journal of Oral and Dental Research
Abbreviated Key Title: Saudi J Oral Dent Res
ISSN 2518-1300 (Print) |ISSN 2518-1297 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: http://scholarsmepub.com/sjodr/

Original Research Article

Antifungal Effect of Spirulina Plantensis in Rat Tongue Mucosa

Sally Hassan Abo Baker*
Ph.D., Lecturer of Oral Biology Department, Faculty of Dentistry, Mansoura University, Egypt

***Corresponding author:** Sally Hassan Abobaker
| **Received:** 02.06.2019 | **Accepted:** 15.06.2019 | **Published:** 21.06.2019
DOI:10.21276/sjodr.2019.4.6.11

Abstract

Aim: to investigate the antifungal effect of spirulina plantensis against candida albicans. Materials and methods: In the present study, we used 60 adult rats weighing between 150 and 200gm. The rats were divided into three equal groups: Group I: Animals served as controls, Group II: Animals were subjected to Candida albicans inoculation on the dorsal surface of the tongue and received systemic antibiotic treatment, Group III: Animals were subjected to Candida albicans inoculation on the dorsal surface of the tongue under systemic antibiotic treatment and then received 15 mg/kg body weight Spirulina platensis extract orally by gastric gavage daily. After 3 weeks, by cervical dislocation, animals of the three different groups were sacrificed. Tongue specimen were dissected from each rat and prepared for histological staining done with Haematoxylin & Eosin and transmission electron microscopic examination. Results: histological examination revealed atrophy of the lingual epithelium and the surface layers was infiltrated with candida yeasts in-group II while in Group III the papillae begin to regain their normal appearance with decreased infiltration of candida to the superficial layers. Ultrastucturely, showed normal appearance of different layers of the lingual epithelium in-group I. Group II: Candida albicans yeasts invaded the keratin layer. The different layers of the lingual epithelium appeared with severely widened intercellular spaces and destructed desmosomal junctions. Group III: keratin layer appeared with disappearance of candida spherical plastopores. The different layers of the lingual epithelium showed less widened intercellular spaces and less destructed desmosomal junctions. Conclusion: Spirulina plantensis can be useful against candida albicans instead of the traditional antifungal drug.

Keywords: Spirulina plantensis, Candida albicans, Transmission electron microscope, Ultrastructure, Animals, Antifungal.

INTRODUCTION

Candidiasis is a fungal infection of any species of candida. The oral mucosa can be infected by several number of Candida species. The most frequently found oral fungal agent is Candida albicans, which is highly infectious due to its higher pathogenicity and adherence properties. Candida albicans is an oral commensal in 40%–65% of healthy adults [1].

The oral cavity could be infect with Candida albicans, which is an opportunistic fungus. Multiple factors can cause increased numbers of colony of Candida albicans in the mouth such as smoking, antibiotics use, immune-compromised condition and weakened immune system [2]. Local inflammation and discomfort in many people can be caused by superficial infections of mucosal membranes and skin by candida [3].

Long-term use of systemic drugs like drugs with xerostomic side effects, broad-spectrum antibiotics, and immune-suppressants drugs, altering the local oral flora, decreasing the salivary flow, disrupting mucosal surface, all this create a suitable environment for candida to grow[4].

Nystatin and Fluconazole are currently the best oral candidiasis medicines. Unfortunately, these drugs might lead to undesirable side effects as; long-term use of fluconazole can lead to resistance, whereas high doses of Nystatin can increase plaque formation and cause gastrointestinal discomfort [5]. Antifungals derived from plants can therefore be a viable oral therapy option for candidiasis. Spirulina Plantensis is one of these potential plants.

A blue-green alga *called Spirulina platensis*, is commonly found in high-salt tropical or subtropical waters but other types can grow in large fresh water lakes [6]. It seems to be a good source of protein in food since it contains 65 to 70% protein. Several studies demonstrate the beneficial effects of *spirulina* on malnutrition treatment and on other pathologies such as

diabetes mellitus, high blood pressure, obesity, and hypercholesterolaemia [7].

Moreover, several studies have shown that different Spirulina platensis extracts can inhibit viral replication and have antimicrobial activity against Gram +ve and Gram -ve bacteria. [8]However, little information is available on Spirulina platensis ' antifungal properties and its potential toxic effects were not studied extensively.

The aim of this study is to investigate the effect of *Spirulina platensis against candida infection by histological and transmission electron microscopic examination.*

MATERIALS AND METHODS

Animals

In the present study, we used 60 adult rats weighing between 150 and 200gm. All procedures involving animals conducted in this study were in accordance with the ethical standards in compliance with the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised, 1978) and have been approved by, Faculty of dentistry, Mansoura University, Egypt. The rats were caged and were supplied by standard rodent's food and provided with water.

Experimental Design: The animals were divided into three equal groups:

- Group I: animals served as controls.
- Group II: animals were subjected to Candida albicans inoculation on the dorsal surface of the tongue and received systemic antibiotic treatment.
- Group III: animals were subjected to Candida albicans inoculation on the dorsal surface of the tongue under systemic antibiotic treatment and then received 15 mg/kg body weight Spirulina platensis extract orally by gastric gavage daily [9]. Spirulina platensis distributed by General Nutrition Corporation GNC.

Antibiotic Therapy

In-groups II, III the rats were given 14 mg/kg body weight of Augmentin in their drinking water twice a day, one week before oral inoculation with C.albicans and continue to receive antibiotic therapy over the experimental period [10]. Augmentin was purchased from GlaxoSmithKline, Egypt.

Oral Candidiasis in Rats

According to Reed *et al*., Candida albicans suspension, containing 5x108 viable cells/ml was prepared [11]. The Candida albicans suspension (0.2 ml) with the aid of a 1ml syringe and 30x8 mm blunt needle was dropped into the rats ' mouth. Then the material was spread with a swab soaked in the suspension previously on the dorsal surface of the tongue. The rats receive no water after inoculation, for at least one hour. This step has been repeated for three consecutive days. Six days after inoculation, Candida albicans infection was tested with sterile cotton applicator by swabbing the inoculated oral cavity, followed by plating on yeast extract- peptone- dextrose agar [12].

After three weeks, by cervical dislocation, animals of the three different groups were sacrificed. Tongue specimen were dissected from each rat. Each specimen was divided into right and left halves. Specimen of the right sides were prepared for histological staining done with Haematoxylin & Eosin while that of the left sides were prepared for transmission electron microscopic examination.

RESULTS

Gross Observations

The tongue dorsal surface of the rats that have been infected with candida albicans and received no treatment showed clinically apparent lesions. It showed patchy areas of smooth mucosa and well-defined atrophic areas.

Light Microscopic Results: (Haematoxylin & Eosin stain):

- Group I: the dorsal surface of the tongue showed normal epithelium with stratified squamous cells and normal architecture of the lamina propria (Fig A, 1).
- Group II: revealed atrophy of the lingual epithelium and the surface layers was infiltrated with candida yeasts. The lamina propria is highly infiltrated with inflammatory cells (Fig A, 2).
- Group III: showed the papillae begin to regain their normal appearance with decreased infiltration of candida albicans to the superficial layers, inflammatory cells were mildly aggregated in the lamina propria (Fig A, 3).

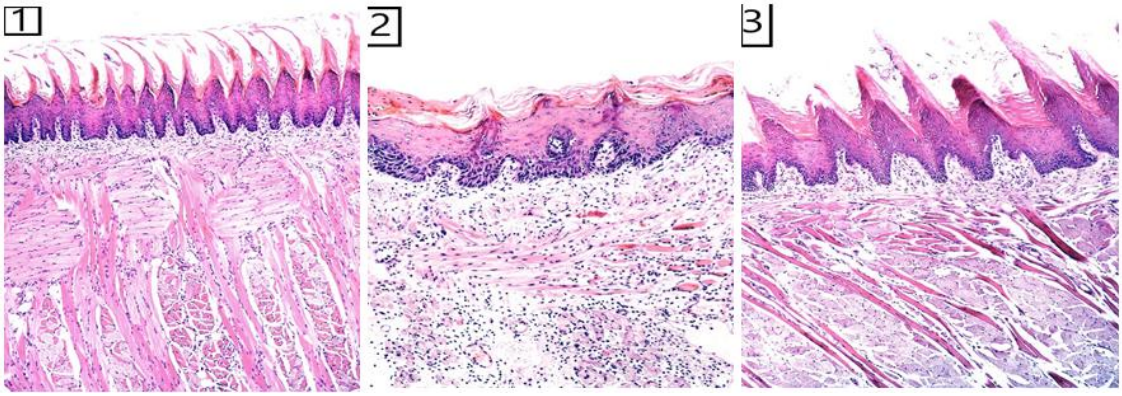

Fig A: (1) group I showed the lingual mucosa with stratified squamous epithelium. (2) Group II showed atrophy of the lingual epithelium with infiltration of the superficial layers with candida yeasts. (3) Group III showing partial disappearance of candidal invasion and the papillae begin to regain their normal appearance. (H&E stain x 100)

Electron Microscopic Results

- Group I: showed normal appearance of different layers of the lingual epithelium with normal intact keratin layer. The granular, spinous, basal cell layers appear normal with euchromatic nuclei, intact desmosomal junctions, and intact basal lamina. {Fig B (1), Fig C (1), Fig D (1), Fig E (1)}.
- Group II: Candida albicans yeasts invaded the keratin layer. The granular, spinous, basal cell layers appeared with severely widened intercellular spaces, destructed desmosomal junctions, and irregular basal lamina. {Fig B (2), Fig C (2), Fig D (2), Fig E (2)}.
- Group III: keratin layer appeared with disappearance of candida spherical plastopores. The granular, spinous, basal cell layers appeared with euchromatic nuclei, less widened intercellular spaces, less destructed desmosomal junctions. {Fig B (3), Fig C (3), Fig D (3), Fig E (3)}.

Fig B: Transmission electron micrograph showed (1) group I with normal intact keratin layer, (2) group II showing Candida albican yeasts appear invading the keratin layer (arrows), (3) group III showing disappearance of Candida albican yeasts from keratin layer.(EM x 17500)

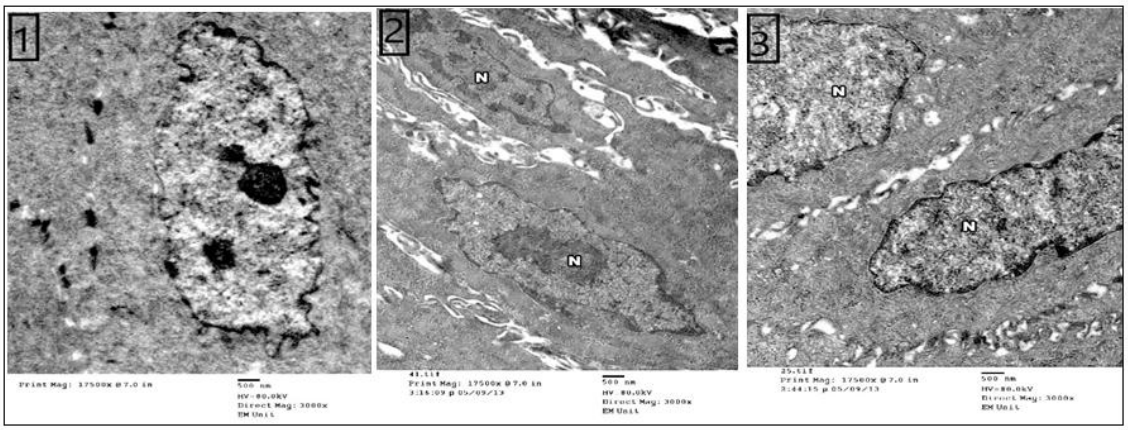

Fig C: Transmission electron micrograph of granular cell layer showing (1) group I with normal looking cells with intact desmosomal junctions, (2) group II the cells appeared with widened intercellular spaces and destructed desmosomal junctions, (3) the cells appeared with less widen intercellular spaces and less destructed desmosomal junctions. (EM x 17500)

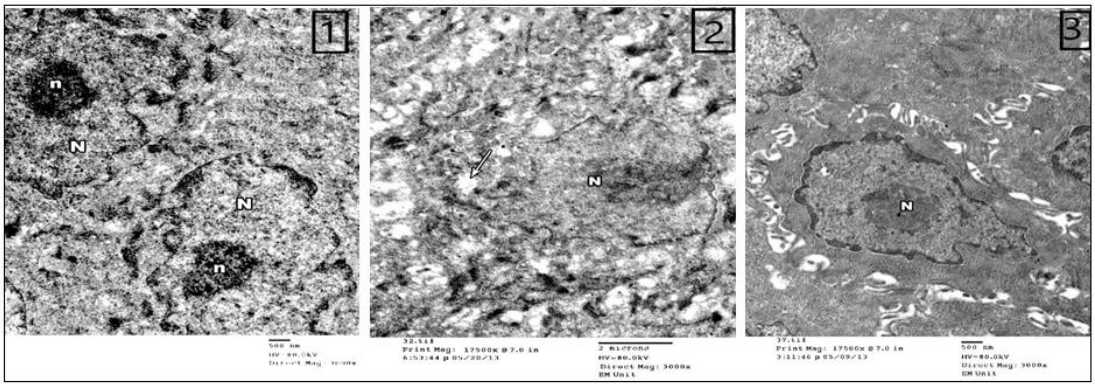

Fig-D: Transmission electron micrograph of spinous cell layer showing (1) group I with normal looking cells with intact desmosomal junctions, euchromatic nuclei (N) and evident nucleoli(n), (2) group II the cells appeared with widened intercellular spaces, destructed desmosomal junctions and swollen mitochondria(arrow), (3) the cells appeared with euchromatic nuclei(N), less widen intercellular spaces and less destructed desmosomal junctions (EM x 17500)

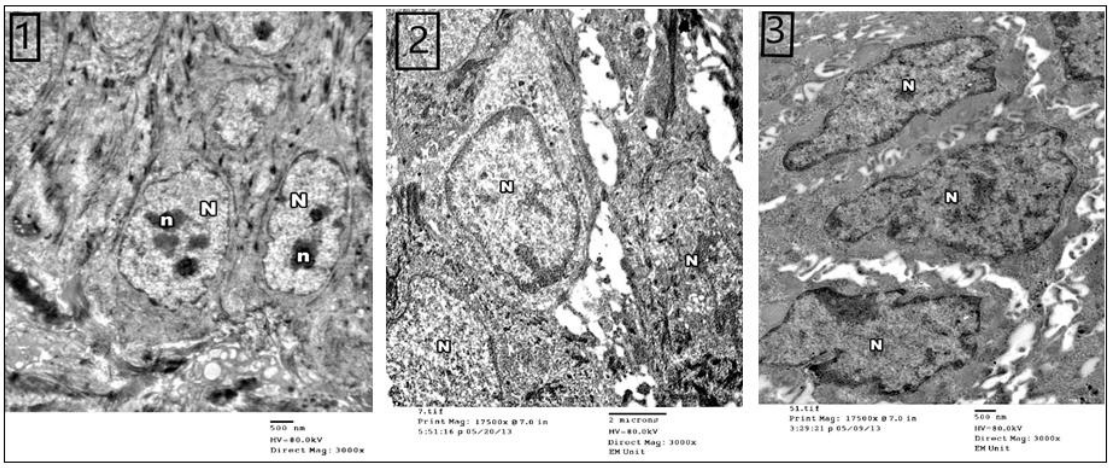

Fig-E: Transmission electron micrograph of basal cell layer showing (1) group I with normal looking cells with intact desmosomal junctions and clear evident nuclei (N) with nucleoli(n), (2) group II the cells appeared with abnormal nucleus(N), widened intercellular spaces and destructed desmosomal junctions, (3) the cells appeared with euchromatic nuclei(N), less widen intercellular spaces and less destructed desmosomal junctions (EM x 17500)

DISCUSSION

Oral candidiasis is one of the common fungal infections that affects the oral mucosa. Candida albicans yeast is the cause of these lesions, which is one of the normal oral microflora components, about 30% to 50% people carry this organism. Increasing the age of the patient will increase the rate of carriage. [13] Oral candidiasis is common among people who smoke, wear dentures, taking antibiotics or corticosteroids, have cancer, diabetes, or HIV infection [14].

To maintain prolonged oral colonization and infection in Sprague-Dawley rats, an Augmentin-laced water and a repeated yeast inoculation were necessary. So Augmentin was used throughout the study [15].

Inoculation of candida albicans suspension in the dorsal surface of tongue of rats in this study result in macroscopic lesions and microscopic alterations in the epithelium and the connective tissue underneath the inoculated area. These results were in agreement with Freire-Garbal *et al.*, [12]; who found that in rats, the candida albicans inoculation would cause lesions in the oral mucosa.

In the present study, after 3 weeks of inoculation of candida albicans in the dorsal surface of tongue in-group II there was atrophy of the lingual papillae with large invasion of tangled candida yeasts masses, our findings were in accordance with Allen & Beck who observed that after 3 weeks of inoculation of candida albicans, there was an epithelial alterations [16].

Ferrer J reported that candida albicans penetrates vertically from the keratinized layer to the superficial layer of the spinous cell layer, and the invasion has three stages [17]:

- Yeast cell Adherence to the epithelium;
- Yeast budding
- Invasion of the epithelium.

As the natural compounds are promising alternatives therapy compared to conventional antifungal medicines, we used spirulina plantensis in the present study. In-group III, after the rats received spirulina plantensis daily there was complete disappearance of candidal yeast and the papillae begin

to regain their normal appearance, these results could be explained according to Antonella Marangoni *et al*., who found that *Spirulina platensis* extract works against all strains of *Candida* [18].

Also Khan *et al.*, stated that Spirulina has many biological functions as antifungal, antibacterial, antiparasite and antiviral activities [19].

The ultrastructure features of the rat tongue mucosa is very close to the healthy human epithelium where the desmosomes provide tight attachment of subjacent cells. For internal support for the epithelium, thick bundles of tonofilaments are found in the cytoplasm of the epithelial cells. After inoculation of the candida albicans on the rat tongue mucosa, group II showed candida albicans yeasts invading kertain layer and different cells of the tongue epithelium showed widen intercellular spaces, destructed desmosomal junction and nuclear changes.

In agreement with our results, Yotaro Abe found that ultrastructually, Candidal hyphe absorbed in the cytoplasmic epithelial vacuole like endocytosis. At the tip of the hypha, destruction of desmosomal cell junction and tonofilament deficiency was noticed also [20].

Also Julie *et al*., reported that with transmission electron microscope, they examine the infected explants and found that fungi was present both extracellular and intracellular throughout the epithelium, they found that candida was often very close to the epithelial cells and showed a close rapprochement between the fungal cell walls and the epithelial cell surfaces [21].

After the rats received spirulina plantensis daily in-group III, ultrastructually the candida yeast disappear from keratin layer and there was less widen intercellular spaces and less destructed desmosomal junctions among different cells of the lingual epithelium. These results is supported by Mostafa M. El-Sheekh *et al*., who stated that from spirulina plantensis, a purified antimicrobial compound is produced which more active against unicellular fungi, *Candida albicans*, Gram negative, and Gram positive bacteria [22].

Also El-Baz FK *et al*., 2013 proved in their study the biological activity of the ethanol extract of Spirulina platensis against Candida albicans, Enterococcus faecalis, DNA enteric and non-enveloped RNA viruses [23].

CONCLUSION

Spirulina plantensis as a natural compound may represent as a promising alternatives therapy compared to conventional antifungal medicines against Candida albicans.

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