Treatment of candidiasis by Ceratonia Siliqua

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Abstract

Candida albicans is the most common fungal pathogen isolated from the oral cavity. The aim of this study was to evaluate the anti fungal activity of the Ceratonia siliqua extract and to observe the effectiveness of the extract in the treatment of oral candidiasis. In vitro study; culture for candidal species was conducted in the lab. identified, and inoculated the agar plate. Different concentrations (1,5,10mg/ml) of Ceratonia siliqua aqueous extract was added and the inhibition zones were recorded after 24 hours of incubation. In vivo study; was conducted in a single-blind technique. Twenty- two patients with oral candidiasis (9 female, 13 males, age range 1 month-60 yrs) were treated by a suspension of 10 mg /ml Ceratonia siliqua extract, applied topically, 4 times daily for 5 days. In vitro results showed no growth of Candida albicans in the agar plate, with different concentrations 1,5,10 mg/ml of Ceratonia siliqua extract while the in vivo study revealed that 18 patients were cured , and 4 patients got partial cure ($p < 0.05$).Our results support the potential and effectiveness of Ceratonia siliqua 10 mg/ml suspension in the treatment of candidiasis with lack of side effects.

Keywords: Ceratonia Siliqua, Candidiasis

Introduction

Candidiasis is an opportunistic fungal infection most commonly caused by the Candida species, Candida albicans. Approximately 60% of healthy children may harbor commensal candidal microorganisms without demonstrating any clinical sign or symptom of mucosal diseased. [(1)]Under a variety of pathologic conditions, Candida can proliferate in the mouth and produce oral lesions. Many predisposing factors have been identified as playing a significant role in the development of oral candidiasis.

Low salivary secretion, poor oral hygiene, removable intra oral prosthesis, inhaled Corticosteroid therapy, chronic antibiotic therapy, diabetes, systemic steroid therapy, immunologic impairment (HIV infection), lymphoma, leukemia, and anemia have all been associated with increased susceptibility for oral candidiasis.[(2،3)] Saliva plays a significant role in oral homeostasis and saliva contains anti microbial proteins including lysozyme, lactoperoxidase, immuno-globulin, histatins, and lactoferrin.[(3)b]Histatins are reported to have potent anti fungal activity and there is also some evidence that salivary IgA inhibits oral adhesion of Candida albicans.[(4)] The salivary calcium-binding myelomunocytic L1 protein, or calprotectin, had also been reported to play a role in the defense against oral candidiasis.[(5)]Candidal infection may occur as superficial white Lesions (pseudo membranous candidiasis), as red patches(erythematous candidiasis), or as a combination of white and red changes on the oral mucosa; angular cheilitis is often associated.[(6)] Prevention of colonization of the oropharynx and prevention of clinical infection (oropharyngeal candidiasis) may be of clinical importance in the prevention of systemic candidiasis and because of the potential for increased morbidity and mortality in neutropenic patients.[(7)]There are many remedies used for Candida albicans; nystatin, clotrimazole, miconazole, econazole and ketoconazole.
Ceratonia siliqua Linne (F. Leguminosae):

Carob gum consists of the endosperm separated from the seeds of certonia siliqua.lt contains mannan, 58%, galactan 29%, pentosans 3%, proteins 5%, cellulose 4% and yields about 0.8% of ash; an oxydase is present and also an enzyme named Ceratoniase.[8] It is used to buffer intestinal contents, adsorb toxins and irritant secretions, regulate hyperperistaltic movement of the intestine and produce formed semi-solid stools, orb thereby tending to eliminate dehydration and electrolyte imbalance.[9] Greally[10] compared the efficacy of the pro-kinetic agent cisapride with that of Gaviscon plus carobel (Carob Seed Flour) in the treatment of gastrooesophageal reflux. Tannin-rich Carob pod (Ceratonia siliqua) is used for the treatment of acute-onset diarrhea of bacterial and viral origin. Indead, Ceratonia siliqua have anti bacterial effect against E.coli. Staph. aureus and α-hemolytic streptococcus [11].However, no data were avialable regarding the antifungal activity of Ceratonia siliqua in the treatment of fungal infection. The aim of this study is to evaluate the effect of Ceratonia siliqua suspension on Candida albicans both in vivo and in vitro.

Material and Methods

In one liter of distilled water 200 g of bud powder from Ceratonia siliqua was dissolved, boiled at 100 C for15min, filtered and autoclaved at 20 C until the extract became dry. A suspension of different concentrations was made.

1-In Vitro Study:

Culture for candidal species was conducted in the laboratory with standard microbiologic technique. Oral cultures were obtained by swab technique of oral mucosa. Candidal species were identified by germ tube evaluation and reported as semiquantitative colony counts were recorded as light, moderate, or heavy growth. The surface of the agar plate is entirely inoculated with 0.1ml of Candida albicans. Individual sterile filter paper impregnated with (1mg/ml, 5mg/ml, and 10mg/ ml) of Ceratonia siliqua water extract, with positive control (which includes filter paper impregnated with chlorhexidine 0.2%) and negative controls (which include filter paper impregnated with distilled water) were used.

Each filter paper was placed equally spaced around the plate. The cultured plates were incubated for twenty-four hours at 37° C, and then the zones of clear medium around the discs were noticed. The inhibition zones were recorded after twenty-four hours of incubation at 37 °C, to evaluate the anti fungal activity of each extract.

2-In Vivo Study:

This study was conducted in a single-blind trial. Twenty-two patients with a diagnosis of candidiasis were included in this study. The diagnosis was based on the oral examination and the presence of a positive laboratory culture. Treatment was supplied by a senior clinician. Patients were instructed to apply topically a suspension of 10 mg/ml Ceratonia siliqua extract 4 times daily for 5 days. Each patient was followed up regularly and daily during the period of application of medication, to determine subjectively the disappearance of infection. The effectiveness of the drug was evaluated after 5 days of treatment by examining the oral mucosa for candidal lesions and documented by examining oral cultures. Statistical analysis was done by chi-square.

Results

1-In Vitro Study:

There is no growth of Candida albicans in the agar plate with wide inhibition zones in different concentrations of Ceratonia siliqua (1,5,10 mg/ml).

Chlorhexidine showed comparable inhibition of growth while distilled water did not impede the growth of Candida albicans

2-In Vivo Study:

Twenty-two patients were studied , 13 male and 9 female , age range from 1 month to 60 years and duration of illness varied from 1 - 14 days. Of the 22 patients, 18 (81.8%) patients were totally cured , 4 (18.2 %) patients were partially cured

• Sex: There were 13 males and 9 females .The percentage of totally cured female patients was 77.8% and partially cured female patients was 22.2%.while for male patients the percentages were 84.6% and 15.4% respectively .By chi-square , the variations in the totally cured and partially cured patients between males and females are significant as shown in table 1.

• Age: Sixteen patients were of an age 6 months and 6

patients were 7 months - 60 years .With patients age of 6 months or less the percentage of patients totally cured was 75% and partially cured was 25% while at age 7 months and more the totally cured was 100% and there was no partial cured . The variations of totally cured and partially cured between the mentioned ages are significant, as shown in table 2 .

Duration of illness: Fourteen patients had the illness for 1 -5 days while 8 patients had it for 6 - 1 4 days . With a duration of illness of 1 - 5 days, the percentage of totally cured was 78,^and the partially cured was 21.4% while those of a duration of illness of 6 - 1 4 days the percentages were 87.5% and 12.5% respectively. By chi-square the variations are significant, as shown in table 3 .

Discussion

Candida albicans is budding yeast ceil in its most common growth form. Its blastospores can start growing hyphae, extending apically with a linear extension rate and branching exponentially as a network.[12] This fungus has been demonstrated to grow in a number of morphologic forms such as germ tubes, blasto spores, pseudo- hyphae, true hyphae, and chlamydospores.[13] All growth patterns except chlamydospores show interconversion to each form of growth depending on the environmental conditions such as pH, temperature, and nutritional source [13] The presence of calcium ions has been shown to have a critical role in the control of this morphogenesis.

Candida albicans can digest dentinal collagen. This will result in release of minerals including calcium, from the crystal phase after the removal of organic content of dentin{14). In vitro, chlorhexidine is active on Gram-positive and Gram- negative bacteria as well as on yeasts. The anti-candida activity of chlorhexidine in vitro is dose dependent and varies with the medium used (15) . However, the use of chlorhexidine failed to affect *Candida* numbers in patients studied(16). Listerine is active against aerobic and anaerobic bacteria as well as *Candida.*

It has been shown to reduce the risk of oral candidiasis but its activity remains low(1/).Some anti-fungal agents such as nystatin, amphotericin B, or miconazole used locally as topical agents contribute to the prevention of systemic Candida infections of oral origin(18). Cyclopirox, an imidazole antimycotic agent and rilopirox, a new hydroxypyridone, significantly inhibit the adherence of Candida albicans to the epithelial cells of the mouth and vagina (19).

Our results showed that boiling water extract of Ceratonia siliqua inhibited the growth of *Candida* in the

agar diffusion assay and prevented the colonization of Candida. The presence of tannins in Ceratonia siliqua was probably the active constituents to adsorb toxins and precipitate protein. Upon hydrolysis arabinose, galactose, glucose, mannose, xylose and various uronic acids are the most frequently observed components of Ceratonia siliqua. The uronic acids may form salts with calcium, magnesium and other cations, methylether and sulfate ester substituents and this further modifies the hydrophilic properties of some natural polysaccharides^. So Ceratonia siliqua may form a salt with calcium and prevent morphogenesis.

Our study clearly demonstrates that the use of Ceratonia siliqua extract effectively cured patients with oral candidiasis even in patients who failed to respond to nystatin. Strains that are able to make a mannoprotein-based fibrillar layer have a considerably increased strength of adhesion to the glycosphingo lipids of the oral epithelial cells. Among the mannoproteins, surface receptors such as the fibrinogen-binding protein and the laminin receptor- like protein possess collagenous domains that mimic the domains of type IV collagen (20) . After adhesion of Candida albicans to the oral epithelial cells, new *Candida* proteins are expressed.

These proteins are phosphorylated acting as a signal for subsequent events[21].Tannins in a solubilized form significantly depressed the protein digestibility by forming an insoluble protein-tannin complex[22]. In this way, the possibility that Ceratonia siliqua tannins interact with Candida phosphorylated proteins prevents subsequent events and as a result eradicates colonization. No toxic effects have been reported with Ceratonia siliqua usage and this probably encouraged people touseitasa safe drug lt is concluded that Ceratonia siliqua extract is effective in the treatment of patients with oral candidiasis being free of side effects.

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Table (1) the effect of Ceratonia siliqua susp. (10 mg / ml) in patients with oral candidiasis: results in patients of both sexes.

Sex	N	PARTIAL	CURED	TOTAL
Male	13	2 15.4%	11 84.6%	13 100%'
Female	9	2 22.2%	7 77.8%	9 100%
Total	22	4	18	22

X^2=5.3 significant PO.05

14. **Table (2**) the effect of Ceratonia siliqua susp. (10 mg / ml) in patients with oral candidiasis: results in patients of different ages.

Age months	N	PARTIAL	CURED	TOTAL
< 6	16	4 25%	12 75%	16 100%
> 7	6	0	6 100%	6 100%
Total	22	4	18	22

X^2 =11.3 significant PO.05

15. **Table (3)** the effect of Ceratonia siliqua susp. (10 mg / ml) in patients with oral candidiasis: results in patients with different duration of illness.

Duration Of illness (days)	N	PARTIAL	CURED	TOTAL
1-5	14	3 21.4%	11 78.6%	14 100%
6-14	8	1 12.5%	7 87.5%	8 100%
Total		4	18	22

X^2=10.8 significant PO.05