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Research Article

COMPARATIVE EFFECT OF MICONAZOLE AND *THYMUS VULGARIS* AGAINST *CANDIDIA ALBICANS* ISOLATED FROM ORAL MUCOSA OF PATIENTS WITH DOWN'S SYNDROME

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ABSTRACT
Candida is a genus of yeasts and is the most common cause of fungal infections worldwide. Many species are harmless commensals or endosymbionts of hosts including humans; however, when mucosal barriers are disrupted or the immune system is compromised they can invade and cause disease. Oral candidiasis is common in elderly denture wearers. Typically, the nucleus of each cell contains 23 pairs of chromosomes, half of which are inherited from each parent. Down syndrome occurs when an individual has a full or partial extra copy of chromosome 21. Down syndrome (DS) or Down's syndrome, also known as trisomy 21, is a genetic disorder caused by the presence of all or part of a third copy of chromosome 21. In these patients, oral anatomical disturbance is most predisposing factor that causes growth and proliferation of these funguses which is seen most commonly in pre-oral. *Thymus vulgaris* (Thyme) is a species of flowering plant in the mint family Lamiaceae, native to southern Europe from the western Mediterranean to southern Italy. In this research we Compared affect of Miconazole and *Thymus vulgaris* against Candidial Species Isolated from Oral Mucosa of Patients with Down's syndrome. used of 53 patients with Down's syndrome who were in the rehabilitation center. Sampling was done using the sterile swaps. For this mean, swaps were stained from 3 areas of oral mucosa-dorsal surface, palate and buccal mucosa. Swaps then were transferred into the tubes with distilled water 0.9% and were shook for separating the funguses from the swaps. After that, samples were transferred into the mycology laboratory. By the results, of 53 cases, 46 (86.79%) of them were positive, so that, 26 (56.52%) and 20 (43.47%) of them were male and female respectively. Of 46 cases, 60 candida funguses were isolated. Of that, 35 cases (58.33%) were C.albicans. MIC50 of miconazole of 11, 18 and 6 cases were 0.25, 0.5 and 2 µg/ml respectively for C.albicans isolates and MIC50 of *Thymus vulgaris* of 6, 8 and 21 cases were 1, 1.5 and 2 µg/ml respectively for C.albicans isolates and the results showed that mean value of MIC for miconazole is less than *Thymus vulgaris* against C.albicans. It means that, candida albicans are more susceptible for miconazole than *Thymus vulgaris*.

Keywords: *Down's Syndrome, Candidia Albicans, Miconazole, Thymus Vulgaris*

INTRODUCTION
Candida is a genus of yeasts and is the most common cause of fungal infections worldwide (Manolakaki *et al.,* 2010). Many species are harmless commensalsor endosymbionts of hosts including humans; however, when mucosal barriers are disrupted or the immune system is compromised they can invade and cause disease (Themistoklis, 2011). *Candida albicans* is the most commonly isolated species, and can cause infections (candidiasis or thrush) in humans and other animals. The genus Candida consist of 200 species that more commonly with *C.albicans, C.dubliniensis, C.tropicalis, C.glabrata, C.parapsilosis* and *C. krusei* are most prevalent and most isolated from lesion of candidiasis. Non-pathogen species of candida such as *C.albicans* are part of natural microflora of the mouth at the range of 17-50% (McCullough *et al.,* 1996). Many species are found in gut flora, including *C. albicans* in mammalian hosts, whereas others live as endosymbionts in insect hosts (Nguyen *et al.,* 2007). Candida is almost

universal on normal adult skin (Jawetz, 1978) and *C.albicans* is part of the normal flora of the mucous membranes of the respiratory, gastrointestinal, and female genital tracts which cause no disease. But overgrowth of several species including albicans can cause superficial infections such as oropharyngeal candidiasis (thrush) and vulvovaginal candidiasis (vaginal candidiasis). Oral candidiasis is common in elderly denture wearers (Darwazeh *et al.*, 1990). In otherwise healthy individuals, these infections can be cured with topical or systemic antifungal medications (commonly over-the-counter antifungal treatments like miconazole or clotrimazole). In debilitated or immunocompromised patients, or if introduced intravenously, candidiasis may become a systemic disease producing abscess, thrombophlebitis, endocarditis, or infections of the eyes or other organs (Enfert *et al.*, 2007). Typically, relatively severe neutropenia is a prerequisite for the Candida to pass through the defenses of the skin and cause disease in deeper tissues; in such cases, mechanical disruption of the infected skin sites is typically a factor in the fungal invasion of the deeper tissues (Goehring, 2008).

In every cell in the human body there is a nucleus, where genetic material is stored in genes. Genes carry the codes responsible for all of our inherited traits and are grouped along rod-like structures called chromosomes. Typically, the nucleus of each cell contains 23 pairs of chromosomes, half of which are inherited from each parent. Down syndrome occurs when an individual has a full or partial extra copy of chromosome 21. Down syndrome (DS) or Down's syndrome, also known as trisomy 21, is a genetic disorder caused by the presence of all or part of a third copy of chromosome 21 (Patterson, 2009). Down syndrome is usually caused by an error in cell division called "nondisjunction." Nondisjunction results in an embryo with three copies of chromosome 21 instead of the usual two. Prior to or at conception, a pair of 21st chromosomes in either the sperm or the egg fails to separate. As the embryo develops, the extra chromosome is replicated in every cell of the body. This type of Down syndrome, which accounts for 95% of cases, is called trisomy 21. Down syndrome can be identified during pregnancy by prenatal screening followed by diagnostic testing, or after birth by direct observation and genetic testing. Since the introduction of screening, pregnancies with the diagnosis are often terminated (Mansfield *et al.*, 1999).

In these patients, oral anatomical disturbance is most predisposing factor that causes growth and proliferation of these funguses which is seen most commonly in pre-oral (Campos, 2001). So, measurement of the antifungal activity of drugs in vitro using the sensitivity testing can be useful to treat patients with the syndrome. There are many routes for measurement the antifungal activity of drugs in vitro that use of broth medium is one of the most common ways (Warnock, 1989). This method is done through four ways such as macro-dilation broth, micro-dilation broth, flowcytometry and calorimetry (Rex *et al.*, 2001).

Thymus vulgaris (Thyme) is a species of flowering plant in the mint family Lamiaceae, native to southern Europe from the western Mediterranean to southern Italy. Growing to 15–30 cm (6–12 in) tall by 40 cm (16 in) wide, it is a bushy, woody-based evergreen sub shrub with small, highly aromatic, grey-green leaves and clusters of purple or pink flowers in early summer. It is very rich in essential oils and these are the active ingredients responsible for most of the medicinal properties. In particular, thyme is valued for its antiseptic and antioxidant properties, it is an excellent tonic and is used in treating respiratory diseases and a variety of other ailments. The plant is used internally in the treatment of dry coughs, whooping cough, bronchitis, bronchial catarrh, asthma, laryngitis, indigestion, gastritis and diarrhea and enuresis in children. Externally, it is used in the treatment of tonsillitis, gum diseases, rheumatism, arthritis and fungal infections. In this research we Compared affect of Miconazole and *Thymus vulgaris* against Candidial Species Isolated from Oral Mucosa of Patients with Down's syndrome.

MATERIALS AND METHODS

In this study we used of 53 patients with Down's syndrome who were in the rehabilitation center. Sampling was done using the sterile swaps. For this mean, swaps were stained from 3 areas of oral mucosa-dorsal surface, palate and buccal mucosa. Swaps then were transferred into the tubes with distilled water 0.9% and were shook for separating the funguses from the swaps. After that, samples were transferred into the mycology laboratory and were cultured on the mediums saboraud dextrose agar with

chloramphenicol and Chrome Candida Agar as linear method for primary and differential diagnosing of the yeast species. Detection of the funguses was made based on several tests such as producing the germ tube, producing the chlamydoconidia on the Corn Meal Agar medium, growth at the 45°C and absorbing the sugar test using the API20C AUX kit and producing factory recommendations.

Preparing the Extract of Thymus vulgaris

For extraction, the plant material washed with water for 30 Minutes and was disinfected with 2% sodium hypochlorite solution. Then to remove residual hypochlorite, rinsed with sterile distilled water and dried and plant material powdered. 50 g of dried powder was soaked in 500 ml of methanol and 48 hours was shaken by shaker. Then by two layers of sterile linen filtered after that centrifuged for 10 min at 9000 rpm and filtered whatman paper number 41 again.

Preparing the Drug Solution

3.2 mg of Miconazole was weighted and was poured into the tubes. Then 5 ml of Dimethyl sulfoxide (DMSO) at the 640 µg/ml were added as solvent. This solution was kept at laboratory condition for half hour then was filtered. Then for measurement the minimum inhibitory concentration (MIC), 1 ml of drug dilution was diluted again with 9 ml distilled water, so, final concentration was gained (64 µg/ml) (John *et al.*, 2008).

Preparing the Fungal Suspension

Suspension from funguses was prepared from each cultured medium then were counted using neubauer slides and light microscope. For this mean, dilution equal with 1×103 cfu/ml was regulated up to level 500 yeasts per each well was obtained after adding this concentration into the each well (John *et al.*, 2008).

Preparing the RPMI 1640 Medium

The powder of RPMI 1640 medium (sigma Co.) was solved in the water and sodium bicarbonates was added at the 2g/L. Then was filtered and transferred into the tubes and were kept in the refrigerator at 4°C. At the time using, 1ml glutamine were adding per 100 ml medium (John *et al.*, 2008).

Experimental Procedures

In this study we used micro-dilution broth method using the 96-well micro-plates. Then for each well, RPMI 1640 were poured. After that, 100µl of Miconazole and extract of *Thymus vulgaris* were added into the first 8 wells and after mixing, 100 Microliter from 1st well were added into the 2nd well, so, serial dilutions were obtained until well 10th. At the end, 100 µl was out. So, drug concentration was 32 and 0.06 µg/ml in the 1st and 10th wells respectively. Then 100 µl of fungal suspension was added to all wells of each row with exception 12^{th} row. Wells of row 11th and 12th were considered as positive control and negative control respectively. Then, micro-plates were incubated for 48 hours at 35°C. At the end, colonies were counted and MIC50 and MIC90 for Miconazole and extract of *Thymus vulgaris* were calculated (John *et al.*, 2008).

RESULTS AND DISCUSSION

Results

Table 1: MIC values obtained from *C.albicans* strains for Miconazole and *Thymus vulgaris*

Treatment	Strain	N	MIC_{50} (µ/ml)
Miconazole	C.albicans	18	0.5
		11	0.25
		6	2
Thymus vulgaris	C.albicans	6	1
		8	1.5
		21	2

In current study, the age range was 4 to 31 years-old. Of 53 patients, 29 (54.7%) were male and 24 (45.2%) were female. of 53 cases, 46 (86.79%) of them were positive, so that, 26 (56.52%) and 20

(43.47%) of them were male and female respectively. Of 46 cases, 60 candida funguses were isolated. Of that, 35 cases (58.33%) were C.albicans (Table 1).

MIC50 of miconazole of 11, 18 and 6 cases were 0.25, 0.5 and 2 µg/ml respectively for C.albicans isolates and MIC50 of *Thymus vulgaris* of 6, 8 and 21 cases were 1, 1.5 and 2 µg/ml respectively for C.albicans isolates (Table 1).

Discussion

Oral candidiasis is an infection of yeast fungi of the genus Candida on the mucous membranes of the mouth. It is frequently caused by *C.albicans*. Oral thrush may refer to candidiasis in the mouths of babies, while if occurring in the mouth or throat of adults it may also be termed candidosis or moniliasis. Newborn babies, Lupus, Diabetics with poorly controlled diabetes, As a side effect of medication, most commonly having taken antibiotics, Inhaled corticosteroids for treatment of lung conditions (e.g., asthma or COPD) may also result in oral candidiasis: the risk may be reduced by regularly rinsing the mouth with water after taking the medication, People with an immune deficiency (e.g. as a result of AIDS/HIV or chemotherapy treatment), Women undergoing hormonal changes, like pregnancy or those on birth control pills, Denture users, Tongue piercing (Yehuda *et al.,* 2010) and Inflammation and whitening of the tongue can also occur due to dryness or environmental irritants such as smoking are the most important risk factors. Down's syndrome favours alterations of the buccal cavity of the children whose bear this chromosomal alteration. Such alterations allow the tissue of the mouth to be populated by Candida yeasts as colonizing and/or pathogenic microorganisms, as in the case of angular cheilitis, in an incidence of 16% of the children (Roncari, 2002). Periodontal disease can be related with microbiological alelobiosis, which includes Candida isolates, due to the formation of dental plaque, low buccal hygiene, neutropenic compromising and repair capacity deficiency present in children with this chromosomal mutation (fast bone loss) (Roncari, 2002). Another factor that would favour the high carriage of Candida in the mouth of children with Down's syndrome is the verification of the physical chemical alterations of saliva secretion. Variation of salivary pH and sodium, calcium and bicarbonate ions concentration, among other substances, seem to affect Candida mouth survival, as it keeps pH oscillation between acidity and alkalinity (Roncari, 2002). It is also added to this chromosomal alteration, the situation of the immune system of children with Down's syndrome. Neutrophils, T lymphocytes and natural killer cells functions are abnormal; the first ones are associated with lower rates of IgG2 and IgG4 immunoglobulins and the others with altered superoxide desmutase favouring the action of Staphylococcus and Candida as common infectious agents in the mouth. The available Candida infections antifungal treatment includes polyene, azole and pyrimidine drugs. Constant findings of Candida yeasts resistant in vitro to these drugs constitute a frequent clinical-laboratory concern. Polyene antibiotics may have their pharmacological action reduced by alterations of the fungal plasmatic membrane lipids composition and the fungal catalase activity increased reducing the sensibility to the oxidative damages of these drugs. The azoles, in general, show lower efficacy against Candida strains due to the low biding affinity of the 14 α demetilase to the antifungal drug, added to the increase of its enzymatic activity and high content of ergosterol in the fungus. On the other hand, the fungal resistance to pyrimidine drugs is probably favored by the loss or mutation of any associated enzymes responsible for its conversion and incorporation to the transcription of fungus RNA. In the case of *C. albicans* strains the nucleic acid constitution affected by mutations, inhibit the production of UMP PP fosforibosil transferase (Ribeiro, 2002).

In a study by Carlstedt *et al.,* 1996 which carried out on 55 cases, it has been revealed that patients with Down's syndrome are more susceptible than normal people (Carlstedt *et al.,* 1996). They showed that colonization of Candida yeasts in 41 cases (74.54%) was more than normal people (25.46%).

Considering this problem that Down's syndrome is one of the most prevalent disorders from buccal point of view, and because of its risk in developing secondary tumors to the mouth, pharynx and esophagus as well as systemic infection, candida infection in order to diagnose and treat patients with this syndrome, special attention should be focused (Amano *et al.,* 2002). However there are so many antifungal drugs, but, because of unknown mechanism of action of fungal diseases and also resistance of some fungus to

specific agents from other hands yield to extend the fungal diseases and hard to control (Dominique *et al.*, 2002).

Achieved results have shown that *Thymus vulgaris* contained antifungal activity against *C. albicans*. Antifungal activity of *Thymus vulgaris* in Akbari study against *C. albicans* isolates Susceptible and resistant of fluconazole showed that *Thymus vulgaris* have prevention effect on *C.albicans* grow in laboratory circumstances (Akbari, 2007). In another article by Pinto and colleagues on the antifungal activity of essential oils (Saliva officialis) against *C. albicans*, only study on effect of this plant in four genus of *C.albicans* that similar results were found (Pinto *et al.*, 2006). In current study MIC50 of *Thymus vulgaris* of 6, 8 and 21 cases were 1, 1.5 and 2 µg/ml respectively for *C.albicans* isolates and *Thymus vulgaris* have effective activity on *C.albicans* isolates of Patients with Down's syndrome.

In present study MIC50 of miconazole of 11, 18 and 6 cases were 0.25, 0.5 and 2 µg/ml respectively for *C.albicans* isolates of Patients with Down's syndrome and the results showed that mean value of MIC for miconazole is less than *Thymus vulgaris* against *C.albicans*. It means that, *C. albicans* are more susceptible for miconazole than *Thymus vulgaris*.

In a research by Hanan *et al.*, 2004 it has been showed that susceptibility of *C.albicans* isolated from oral cavity of patients with cancer to azolic antifungal agents such as ketoconazole and fluconazole was 1, 0.125 and 1-8 µg/ml and was 1-2 and 2-8 µg/ml about *C.glabrata*, it shows more susceptibility of candida species to ketoconazole than fluconazole, which is compatible with our research's results (Hanan *et al.*, 2004). In another research by Hamza *et al.*, 2008 it has been declared that candida species isolated from oral cavity of patients with HIV have more susceptibility to azolic agents which is compatible with our research results (Hamza *et al.*, 2008).

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