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Research Article

EVALUATION OF CINNAMON MOUTHWASH ON CANDIDA ALBICANS IN TYPE II DIABETICS WITH CHRONIC PERIODONTITIS: A PILOT STUDY

[1,*]Dr. Sheela Kumar Gujjari, [2]Dr. Shreeshyla HS, [3]Dr. J Suresh and [4]Dr. Venkatesh, M.P.

[1,3]Department of Periodontology, JSS Dental College and Hospital, Mysuru, Karnataka, India
[2]Department of Oral Pathology and Microbiology, JSS Dental College and Hospital, Mysuru, Karnataka, India
[4]Department of Pharmaceutics, JSS College of Pharmacy, Mysuru, Karnataka, India

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ABSTRACT

Background and objectives: The aim of the study is to assess the candidial carriage in type II diabetes patients with chronic periodontitis according to the duration of diabetes,gender and age also to evaluate the effect of cinnamon mouthwash on candida albicans in type II diabetic patients with chronic periodontitis.Exfoliative cytology was used as a screening tool for detecting candida albicans.
Material and methods: 30 patients were divided into 2 groups that is diabetic type II patients with chronic periodontitis (test) and non-diabetic patients with chronic periodontitis(control). Glycosylated haemoglobin, questionnaire regarding duration of diabetes and medications were recorded and exfoliative cytology was carried out to detect candida albicans. Patients with candida albicans positive were taken up for the study. Both groups underwent scaling and root planing .On completion freshly prepared cinnamon mouthwash was dispensed in 100ml bottles with instruction for usage. At end of 3months exfoliative cytology was done and patients were advised to stop usage of mouthwash one week after cessation of mouthwash patients were recalled for clinical examination and exfoliative cytology.
Results: Candidal carriage was more in diabetics than non diabetics with chronic periodontitis. There was no difference between gender and the candidal carriage was more in patients with longer duration of diabetes over 10 years and severity of candidal carriage was more according to the duration. Candidal carriage was found to be more in uncontrolled diabetics. There was significant reduction in candidal carriage between baseline and 3 weeks in diabetic patients and in non-diabetics. After cessation of mouthwash recolonisation of candida albicans was observed in both the groups.
Conclusion: Results of the study proved the potential of cinnamon as an anti-fungal agent.

INTRODUCTION

Patients with diabetes mellitus have been found to have increased frequency of oral candidal carriage and increased risk of candidiasis, which is relevant to poor metabolic control, neutrophil dysfunction, reduced salivary flow, high blood glucose in blood and saliva and in medication (Lamichhane, 2015). There are reports indicating that higher glucose levels in saliva of diabetic patients could predispose to oral candidial infection. Candidal colonization can be confirmed by various methods. The most common being culture, but recently exfoliative cytology has been found to be effective in identifying these organisms (Bakthavatchalam Pallavan, 2014).

****Corresponding author: Dr. Sheela Kumar Gujjari,***
Department of Periodontology, JSS Dental College and Hospital, Mysuru, Karnataka, India.

Cinnamon, a commonly used spice which has been used since ancient times, has been found to have medicinal benefits in addition to its flavouring property as a condiment. The bark of *C. zeylanicum and C. cassia* are the only two approved medicinal herbs of the genus *Cinnamomum.* It has been found to have anti-inflammatory, antibacterial, antioxidant properties as well as antifungal (Robert, 2013). Though there are reports of cinnamon being given as an antifungal in HIV patients there isn't sufficient evidence on the antifungal property. Increased frequency of Oral Candidasis has been seen in patients with Diabetes Mellitus and Chronic Periodontitis. The main reservoir for candida in the oral cavity has been found to be the buccal mucosa and tongue. These organisms can also coaggregate with the bacteria in the subgingival biofilm and adhere to the epithelial cells, thereby invading the gingival connective tissue. Prevelance in subgingival periodontal pocket of chronic periodontitis patients has been found to be 76.2%

(Janaina, 2010). More than 90% of fungemia is attributed to candida species and has important implications in mortality. Emergence of resistance to candida is commonly seen with the currently available antifungal drugs.As diabetics are more prone to fungal infections,it is necessary to study the candidial carriage in different diabetic patients depending on their age, sex, duration and glycemic control. Cinnamon, being a herbal product has lesser systemic side effects. As there is limited evidence on the antifungal property, this study was carried out to evaluate the effect of cinnamon mouthwash on Candida albicans in diabetic patients with chronic periodontitis.

MATERIAL AND METHODS

Ethical approval was obtained from the Institutional review board ethical committee governing the use of human subjects in clinical trial JSS Dental College and Hospital and affiliated institution of Jagadguru Sri Shivarathreeshwara University, Mysuru. The trial was a parallel interventional pilot study conducted over a period of 1 month. The inclusion criteria for study group were Patients above 40yrs with clinically healthy oral mucosa, Medical history of type II diabetes mellitus for a minimum period of 1 year irrespective of whether they were on any medication of diabetes or not. Control group consisted of healthy individuals without any systemic illness. Both study and control groups were patients with presence chronic periodontites, who had atleast 20 teeth, probing depth >5mm and who were willing to be a part of the study were selected. In both study and control group candida should be present. Before beginning of the study informed consent was obtained from all the study participants. The diabetics were monitored by the physician and information was obtained regarding type of medication and duration of diabetes. Total of 30 patients were selected based on inclusion and exclusion criteria and were equally divided into study and control groups. In both the groups mucosal scrapings were taken from the dorsum of the tongue and buccal mucosa and sent to Department of Oral Pathology and Microbiology of JSS Dental College and Hospital for Exfoliative cytology to detect the presence of Candida albicans. The subjects of both the groups underwent scaling and root planing. Cinnamon was extracted and mouthwash was prepared in JSS College of Pharmacy. The mouthwash was prepared based on the MIC (Minimum Inhibitory Concentration) which was determined in the Microbiology Department of Life Sciences, JSS University. The mouthwash was dispensed in sterilized 100ml containers.
At the end of scaling and root planing the patients were given oral hygiene instructions and were directed to use 10ml of mouthwash twice daily for 1min for a period of 3weeks.The patients were periodically monitored for any adverse reactions. At the end of 3weeks clinical examination was done and candida albicans levels were evaluated. During 4th week mouth wash was not given to patients. At the end of 4th week candida albicans again evaluated to see the effect of cinnamon mouth wash after cessation.

Experimental

Preparation of cinnamon extract

An aqueous cinnamon extract was prepared using the Soxhlet technique[5] from the barks of *Cinnamomumzylinicum* from Kollam district, Kerala as verified by Dr M. N. Naganandini, Assistant Professor, J.S.S College of Pharmacy, Mysuru.

Plant Profile

Habitat: The spice is derived from the brown bark, which forms quills with longitudinal striations. The plant is native to Sri Lanka, South eastern India, Indonesia, South America, and the West Indies.

Plant Hierarchy

Kingdom *Plantae* – Plants
Subkingdom *Tracheobionta* – Vascular plants
Superdivision *Spermatophyta* – Seed plants
Division *Magnoliophyta* – Flowering plants
Class *Magnoliopsida* – Dicotyledons
Subclass *Magnoliidae*
Order *Laurales*
Family *Lauraceae* – Laurel family
Genus *Cinnamomum* Schaeff. – cinnamon P
Species *Cinnamomum zylinicum* J. Presl – cinnamon P

Scientific Names

Cinnamomumzylinicum, Cinnamomum cassia,
Cinnamomumzeylanicum, Cinnamomumloureirii

Common name(s)

Cinnamon, Cinnamomon, Ceylon cinnamon,
Chinese cinnamon, Chinese cassia, Saigon cinnamon

Phytochemistry

One of the most popular spices used worldwide, cinnamon is known for its aromatic fragrance and sweet, warm taste. The spice is derived from the bark of an evergreen tree, which belongs to the family *Lauraceae*. Mostly cinnamon is used as a spice, cinnamon has great medicinal value. It has been found to be extremely helpful in the treatment of Type 2 diabetes mellitus and insulin resistance. The scientific studies have proved that a variety of biologically active chemicals have been found in Cinnamon which have immense medical potential. It contains a number of compounds, including essential oils that provide the spice's flavor. Other compounds which are present in lesser percentages those are Cinnamic acid, Hydroxyl Cinnamaldehyde, Cinnamyl alcohol, Coumarin, Cinnamyl acetate, Borneol etc.

Preparation of Cinnamon extract

An aqueous cinnamon extract was prepared using the Soxhlet technique from the barks of Cinnamomumzylinicum from Kollam district, Kerala as verified by Dr M. N. Naganandini, Assistant Professor, J.S.S College of Pharmacy, Mysuru. 25g of coarsely ground cinnamon powder was added to 150 ml of distilled water in a round bottom flask. Porcelain pieces were added to the mixture to avoid bumping of liquid during boiling. The flask was then clamped into a ring water bath, with a soxhlet apparatus (condenser) attached to it. The condenser was used to avoid any water loss, as it condenses the water vapour released from the mixture back into the flask. The temperature of the water bath was set to 100 C till vapourarised and then reduced to 50 C – 60 C and maintained till complete extract was obtained. After the flask cooled down, it was unmounted from the water bath and the liquid extract was

poured into a petridish. The liquid extract was then concentrated by placing the petridish in an electric water bath at 40 C till complete water content was lost. The dried extract was then scraped out and collected in sterile containers. This process was repeated until adequate amount of extract was obtained. The mouthwash was prepared based on the MIC which was determined in the Microbiology Department of Life Sciences, JSS University.

MIC Detection

Stock solution was made by dissolving 100mg of cinnamon extract in 10ml of polyethylene glycol. Different concentration were made by taking 50mg of extract for 0.5%,40mg for 0.4%,20mg for 0.2%,10mg for 0.1% and 2.5mg for 0.025%.Antifungal solution was made by dissolving fluconazole tablet in water. Candida was inoculated over the nutrient agar in petriplates.After solidification of the media; 3wells were made using cork-borer in each petriplates. One well was filled with cinnamon extract in different concentrations, one well was filled with antifungal solution and the third well was filled with PEG 400 solution. All the petriplates were kept in a tray for 24 hours. After 24 hours the zone of inhibition and clear zone were measured using a ruler. No zone was noted around Peg 400well.The MIC was found to be maximum at 0.5%.Using the 0.5% concentration the mouthwashes were formulated.

Determination of Minimum Inhibitory Concentration (MIC) based on the zone of inhibition

Concentration (in %)	Diameter of inhibition zone in plant extract (in cm)	Diameter of inhibition zone in antifungal (in cm)
1	3.2	2.9
0.5	2.3	1.2
0.4	2.7	4
0.2	1	2
0.1	1.7	2.6
0.05	1.5	2.1
0.025	2.5	1.9

RESULTS AND DISCUSSION

Results

Table 1. Comparison of candidal carriage among diabetic and non-diabetic patients

Groups	Mild n (%)	Moderate (%)	Severe (%)	Chi square value	P value
Diabetic	3(20)	6(40)	6(40)	9.000	0.011(sig)
Non-diabetic					

Table 2. Assessment of candidal carriage among diabetics according to duration and age

<10 years	3(20)	3(20)	6(60)	5.625	0.06 NS
> 10 years	0(0)	3(100)	0(0)		

Table 3. Candidal carriage according to the glycated levels among diabetic patients

Controlled	0(0)	3(100)	0(0)	5.625	0.060 NS
Uncontrolled	3(25)	3(25)	6(50)		

Table 4. Usage of mouthwash for 3weeks

	nil	mild	moderate	severe		
Baseline	0(0)	3(20)	6(40)	6(40)	22.00	0.000(sig)
3 weeks	9(60)	6(40)	0(0)	0(0)		

Table 5. Effect of cinnamon mouthwash after stoppage

Comparison of candidal carriage between baseline to 4wks among diabetics						
	nil	mild	moderate	severe		
Baseline	0(0)	3(20)	6(40)	6(40)	20.00	0.00(sig)
3 weeks	0(0)	15(100)	0(0)	0(0)		

Table 6. Effect of cinnamon mouthwash after stoppage

Comparison of candidal carriage between baseline to 4weeks among non-diabetic patients						
	nil	mild	moderate	severe		
baseline	0(0)	9(60)	6(40)	0(0)	20.00	0.00(sig)
3 weeks	0(0)	15(100)	0(0)	0(0)		

Table 7. Comparison of candidal carriage among non diabetics according to gender

	mild	moderate	severe		
male	6(40)	0(0)	0(0)	6.67	0.017(sig)
female	3(20)	6(40)	0(0)		

Table 8. Usage of mouthwash for 3wks

Comparison of candidal carriage between baseline to 3wks among non-diabetic patients				
	nil	mild	moderate	severe
baseline	0(0)	9(60)	6(40)	0(0)
3 weeks	9(60)	6(40)	0(0)	0(0)

DISCUSSION

Candida are opportunistic pathogens that can cause disease in host who are compromised by underlying local or systemic pathological processes. Changes in the cellular and humoral immune response may allow different species such as Candida to colonize the sub gingival environment. It has been reported that the proportion of yeast in the periodontal pocket is similar to some bacterial periodonto pathogens suggesting a possible role of Candida in the pathogenesis of the disease. Studies report that candida albicans carriage and density are increased in the oral cavities of diabetic patients and that yeasts are elevated in the periodontal pocket of diabetic patients. In this study also prevalence of candida was more in the diabetic than non diabetics. Candida is the most common yeast found in the oral cavity. Existing mainly in two morphologic forms Budding yeast (blastphore innocuous) and Hyphal (responsible for pathogenesis) The adhesion of the C.albicans to buccal epithelial cells have been reported and the common sites of colonization are buccal mucusa, dorsum of the tongue, tonsillar region. Hence exfoliative cytology was carried out in this study which helpd in morphologic identification. Commonly used method are culture and bio chemical tests, which is more time consuming and expensive. Exfoliative cytology proved to be a useful tool to identify the organism.Chronic periodontitis patients with diabetes showed increased conversion of yeast to hyphal forms but this technique may not be useful to differentiate other species of candida for which molecular techniques are required. Most of studies have found gender and sex to effect the candidal carriage Males exhibited > females

But in this study no significance was observed. In some studies it has been seen that Individuals over the age of 40 exhibited increased levels of candidal infections compared to younger patients .In this study age also did not affect the carriage which is in agreement with Lamey et al who observed that .Factors such as age ,sex,duration of diabetes did not have any relation with degree of oral candidal carriage Present study patients with well controlled diabetes HbA1c <6 compared to subjects with poor diabetic control (HbA1C >9) candidal infections were more but not statistically significant.Other studies in agreement with Mubarak et al Cinnamon in addition to its popularity as a spice has been found to have medicinal properties such as anti-bacterial, anti-fungal, anti-oxidant. As fungi is also found to play a role in the pathogenesis of periodontitis as well as oral lesions in diabetics,cinnamon's role as anti-fungal has been explored in this study. It was used for 3 weeks by both the groups and evaluation was done at the end of 1 week after the stoppage of the mouthwash. Till date no such studies have been reported in this regard. There was a significant reduction in candida from the baseline to 3 weeks in both the groups with more significant result groups in diabetic group with periodontitis 1 week after stoppage .It was seen that even though the candida had decreased there was a tendency of the organisms to recolonize So, it was effective in the severe cases.

Conclusion

The treatment for periodontal disease include scaling , root planing with proper oral hygiene but it may fail to eliminate reservoirs of infectious organisms or the appearance of super infecting pathogens like Candida species Anti-fungal can be used but most of these organisms are resistant to present anti-fungal and anti- microbial. So, it is necessary to seek new anti-fungal agents which can cause disruption or destruction of biofilms and which are effective in isolates that express resistance. Plants have been used in medicine for a long time as they are economic, easily available and applicable in various diseases. One such herb is cinnamon. The results of this study show that cinnamon could be a promising alternative anti-fungal.

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Declaration of Interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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