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Research Article

ORANGE PEEL MOUTHWASH-AN EFFECTIVE TOOL AGAINST CANDIDA IN DIABETIC PATIENTS-A Pilot Study

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A R T I C L E I N F O

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Chlorhexidine, candidiasis, diabetes mellitus, orange peel extract

A B S T R A C T

Context: Diabetes mellitus increases susceptibility to candidiasis for which chlorhexidine has proven to be effective but has side effects like staining, dysgeusia. So, citrus aurantium (bitter orange) a natural product with no known side effects can be used to prevent candidiasis in diabetes patients as it is proven to be effective in various oral diseases.

Aims: To evaluate the efficacy of orange peel extract mouthwash when compared with 0.2% Chlorhexidine mouthwash and distilled water on oral candida albicans (CA) in type II diabetic patients.

Methods and Material: 15 type-II diabetic patients were randomly divided into 3 groups of 5 each. Group 1-Distilled water, Group 2-0.2% chlorhexidine mouthwash and Group 3-Orange peel extract mouthwash. Swab test was done before and after the use of distilled water and mouthwashes.

Statistical analysis used: Kruskal Wallis ANOVA and Spss version 22.0

Results: Respective mouthwashes when compared with distilled water (placebo) showed statistically significant decreased candidal count when compared to placebo. Both the mouth washes were effective in decreasing the CA count, which was statistically significant ($p > 0.05$).when mouthwashes were compared results stated that they were equally potent and no statistical significance was noted.

Conclusions: Orange peel extract mouthwash was found to be as effective as chlorhexidine mouthwash in reduction of CA whereas placebo showed no decrease in CA count.

INTRODUCTION

Diabetes Mellitus (DM) is the most prevalent global health problem causing multiple organ dysfunction. It causes oral problems like periodontitis, taste alterations and increases the prevalence of fungal infection like candidiasis by 80%.[1, 2]Topical and systemic antifungal agents are used in treatment of oral candidiasis but over a period of time resistance to drugs develops and stopping of medications results in higher recurrence rate of candidiasis.[3]Hence, prevention should be the main goal than treatment of oral candidiasis.

Chlorhexidine mouthwash prevents candidiasis, but its long term use causes side effects like discolouration of the teeth, restorative materials and dorsum of tongue, dysgeusia and hypersensitivity which ranges from localized urticaria to anaphylactic shock.[4, 5]Hence, a mouthwash with minimal side effects and as an effective as chlorhexidine is required to prevent candidiasis.

Citrus aurantium (bitter orange) is FDA approved drug used in various diseases such as stomach ache, hypertension, Scurvy.[6] In-vitro studies conducted on Citrus aurantium (bitter orange) has been proved to be effective against candidiasis. Hence an in vivo study was undertaken to evaluate the efficacy of Citrus aurantium (bitter orange) in prevention of candidiasis in type 2 diabetic patients.

A plant Citrus aurantium (bitter orange) belonging to family Rutaceae is used in treatment of various oral diseases as it is a natural product with no known side effects. The orange peel contains flavonoids, vitamin C, carotene, coumarins, triterpenes, and pectin. The flavonoids have anti-fungal, anti-inflammatory and antibacterial properties.[6]

The aim of the study was to evaluate the efficiency of Orange Peel Mouthwash on oral candida albicans in type II diabetic patients by comparing it with chlorhexidine mouthwash and distilled water.

This is the first in-vivo study conducted to evaluate the efficacy of Orange peel extract mouthwash against Candida albicans in type II diabetes patients.

SUBJECTS AND METHODS

The ethical committee of The Oxford Dental College and Hospital, registered on 17.11.2017. The Institutional Ethics Committee granted ethical committee clearance 258/2017-18 for the study on TO EVALUATE THE efficiency of orange

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peel extract mouthwash on oral candida albicans in type-2 diabetic patients**.**

A pilot study was conducted on 15 type II diabetic patients who were randomly divided into 3 groups of 5 each.

Group 1-(placebo) distilled water
Group 2- 0.2% chlorhexidine mouthwash
Group 3- orange peel extract mouthwash.

Participants included were Type II Diabetic patients with no other systemic diseases. Pregnant patients, smokers and alcoholics, patients with other systemic diseases, subjects with hypersensitivity or allergy to the study medications were excluded from study

METHODS

Prior to starting the study, ethical clearance was obtained from the ethical board of the Oxford Dental College. An informed written consent from the patients was taken.

15 type II diabetic patients who fulfilled the inclusion criteria were randomly divided into 3 groups of 5 each.

Group 1-(placebo) distilled water
Group 2- 0.2% chlorhexidine mouthwash (active ingredient-0.2% chlorhexidine gluconate solution)
Group 3- orange peel extract mouthwash (active ingredient-4% bitter orange extract v/v)

Case history was recorded and haematological investigations (RBS-Random Blood Sugar) and glycosated haemoglobin (HbA1c) were measured using fully automated H.P.L.C (High Performance Liquid Chromatography) in laboratory to confirm diabetes. Swab test was performed on all surfaces of the oral cavity (buccal mucosa, anterior and posterior teeth and dorsum of tongue) before and after the use of placebo in group 1, Chlorhexidine mouthwash in group 2 and orange peel extract mouthwash in group 3. 10 ml of placebo was dispensed to patient and instructed to swish for 30sec and spit in group 1.10 ml of 0.2% chlorhexidine mouthwash was dispensed and the patients were instructed to swish the mouth for 30 sec and spit in group 2. 10 ml of orange peel extract mouthwash was dispensed and patients were instructed to swish for 30 sec in group 3, within 10 minutes the swab test was performed in group 1, group 2 and group 3 patients and collected samples were delivered to laboratory.

In laboratory microbial culture in Sabouraud's Dextrose agar (SDA) was performed by sterilizing SDA by autoclaving at 121^{o}C and at 15 lbs for 15 minutes. In a sterilized Laminar Air Flow chamber, approximately 20 ml of the prepared media was poured into sterile disposable petri plates and allowed to solidify. The collected (saliva) samples were then serially diluted to 10^{-5} using sterile water. Since the bacterial load of the sample was unknown, 100µl of the undiluted sample, 100 µl of 10^{-2} and 10^{-5} dilutions were inoculated on the media by spread plate technique and incubated at 37^{o}C for 48 hours. The creamy white coloured smooth and pasty appearance colonies on each plate were counted and tabulated.

RESULTS

Results were calculated using Kruskal Wallis ANOVA and Spss version 22.0.

The mean age of the patients in GROUP 1 was 40.8±11.5, GROUP 2 is 48.2±6.4 and GROUP 3 was 55.2±6.4.Distribution of the number of cfu of candida albicans at baseline and post intervention period in Group 1, 2, 3 shows statistically significant decrease in the number of colony forming unit (cfu) in group 2 and 3 after the use of mouthwash but no statistical difference were seen in group 1(Table 1).

Comparison of mean candida colony forming unit after the use of placebo and chlorhexidine mouthwash between group 1 & 2 respectively shows statistical difference proving chlorhexidine group to be effective when compared to placebo (Table 2).

Comparison of mean candida colony forming unit after the use of placebo and orange peel extract mouthwash between group 1 & 3 respectively shows statistical difference proving orange peel extract group to be effective when compared to placebo (Table 3).

When comparing the mean differences of both the groups, group 2 showed higher reduction of colony count than group 3 with no statistical significant difference noted (p-0.05) indicating the candida count reduction seen in both groups is the same, hence chlorhexidine and orange peel extract mouthwash have the same effectiveness in reducing candida albicans colony count (Table 4).

Comparison of mean candida colony forming unit in group 1, and 3 shows statistical difference between group 1 and 2 , 1 and 3 but no statistical difference between 2 and 3.(Table 5).

Table 1 Distribution of the number of cfu of candida albicans at baseline and post intervention period

	Group 1	Group 2	GROUP 3
Baseline	244.8±11.6	135.4±207.2	41.8±27.2
Post intervention	213.8±38.7	21.8±11.7	16.1±14.3
Z value	1.6	2.3	2.2
p value	0.1	0.04*	0.04*

Table 2 comparison of mean candida (post) between group I & II

	Candida (post) Mean±sd	Z	P
GROUP I	213.8±38.7	2.61	0.009*
GROUP II	21.8±11.7		

p value significant at 0.05

Table 3 comparison of mean candida(post) between group I &III

	CANDIDA (POST) Mean±SD	Z	P
GROUP I	213.8±38.7		
GROUP III	16.1±14.3	2.7	0.006*

p value significant at 0.05

Table 4 comparison of mean candida (post) between group II &III

	CANDIDA (POST) Mean±SD	Z	P
GROUP II	21.8±11.7	0.7	0.4
GROUP III	16.1±14.3		

p value significant at 0.05

Table 5 comparison of mean candida(post) among the study population

GROUP	CANDIDA (POST) Mean±SD	H	p
Group I	213.8±38.7		
Group II	21.8±11.7	9.6	0.008*
Group III	16.1±14.3		

DISCUSSION

Chlorhexidine mouthwash is considered as a broad spectrum antimicrobial agent as it not only affects bacteria but also reduces CA growth in oral cavity by suppressing the ability of CA to adhere to buccal epithelial cells in an individual. Chlorhexidine mouthwash also has ability to reduce cell surface hydrophobicity in CA thus, preventing growth of CA.[7] In an in-vitro study done by Shino et.al antimicrobial activity of 0.2% Chlorhexidine, Coconut Oil, Probiotics, and Ketoconazole were compared to evaluate its effect on Candida albicans in Children with early childhood caries and concluded that chlorhexidine is equally effective as that of ketoconazole.[8] Aoun G et.al compared 0.12% chlorhexidine (CHX) digluconate with 0.05% cetylpyridinium chloride (CPC) in eliminating Candida albicans colonies in dentures and concluded that CHX proved useful in disinfection of the dentures as it reduces the candida colonies.[9]

An invitro study was conducted by Nazhvani et.al to determine the prevalence of candida colonization in oral cavity of liver transplant patients and different exposure times to common mouthwashes and concluded that susceptibility tests of the Candida species to 0.2% chlorhexidine is more at the exposure time of 60 seconds.[10]

In our study 0.2% chlorhexidine was found to be effective in reduction of CA within 15 minutes of its application while (placebo) distilled water used during pilot study was proved to be ineffective against CA thus eliminating the bias of mechanical effect of rinsing the mouth.

In an in-vitro study conducted by Trabelsi D et.al the Chemical constituents present in orange peel extract like Limonene, Linalool, α-Terpineol has retention time of 3-14 minutes after application.[11] In our study orange peel extract mouthwash has proved to be effective in reducing CA within 15 minutes of application.

In an in-vitro study conducted by S. Abhay et.al where a polyherbal toothpaste was formulated using hydroalcoholic extracts of apple peel, lemon peel, orange peel, banana peel and it was proved to be effective against gram-positive bacteria Staphylococcus aureus, Enterococcus faecalis, Streptococcus mutans and gram-negative bacteria Porphyromonas gingivalis.[12]

In-vitro study conducted by Oliveira et.al to evaluate the antimicrobial effects of Citrus limonum and Citrus aurantium essential oils on biofilms formed by Candida albicans, Enterococcus faecalis and Escherichia coli by comparing it with 0.2% chlorhexidine (CHX) and 1% sodium hypochlorite (NaOCl) on multispecies and concluded that Citrus limonum and Citrus aurantium are effective against biofilms formed by Candida albicans, Enterococcus faecalis and Escherichia coli.[13]

An in-vitro study conducted by Sapna et.al in 2016 to study the antimicrobial effects of Citrus sinensis peel extracts (orange peel extract) against bacteria like Streptococcus mutans and Lactobacillus acidophilus causing dental caries and concluded that orange peel extract proved to be effective against them as Citrus sinensis peel extracts contains compounds with therapeutic potential, thus an in vivo clinical studies should be conducted to determine the exact dosages and its effectiveness.[14]

Several in vitro studies concluded that orange peel extract was effective in reduction of candida albicans, an in vivo study was conducted to evaluate the efficacy of orange peel extract. A randomized control clinical trial was conducted by Shanbhog et.al in 2015 to evaluate the efficacy of 0.2% chlorhexidine gluconate and 2% lemon mouthwashes (citrus fruit) on the growth of oral candidiasis in elderly long standing diabetic population and authors concluded that chlorhexidine mouthwash showed better results than lemon mouthwash, our study showed same efficacy between the orange peel extract mouthwash and 0.2 % chlorhexidine and it can act as an alternative herbal formulation which is natural and effective against C albicans.[3]

In this study citrus fruit lemon extract is used, since bitter orange is also a citrus fruit this study can be taken into consideration. In our study orange peel mouthwash was compared to chlorhexidine mouthwash and it was concluded that both the mouthwashes were equally effective against C.albicans.

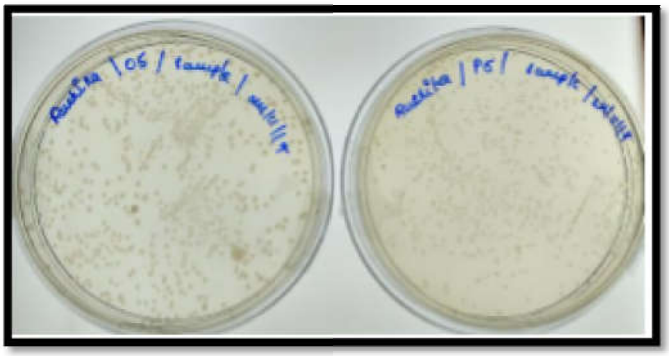

Figure 1 shows the microbial culture before & after the use of distilled water

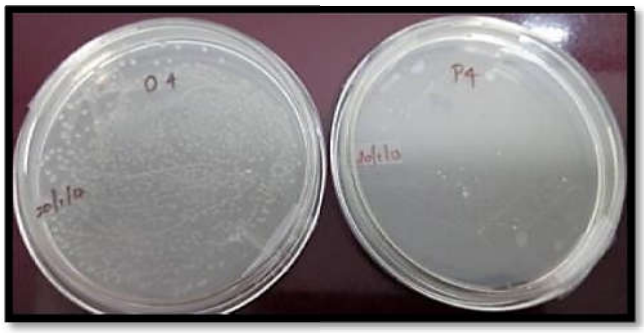

Figure 2 shows the microbial culture before & after the use of orange peel extract mouthwash

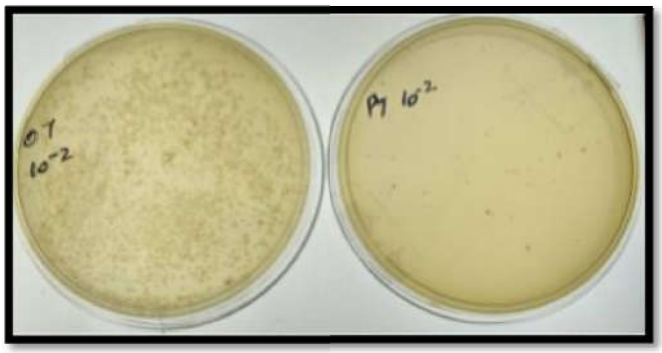

Figure 3 shows the microbial culture before & after the use of Chlorhexidine mouthwash

CONCLUSION

Diabetes Mellitus (DM) causes various changes oral microflora which also increases the candida albicans colonies

causing an opportunistic infection to prevent this infection chlorhexidine is proved to be effective but it causes hypersensitivity reaction and also its long term use results in permanent staining of teeth and dysgeusia hence Orange peel extract mouthwash can be used, as it is a natural and safe herbal formulation proven to equally effective as chlorhexidine mouthwash in reduction of CA and can be consider as a promising antifungal agent to prevent candidiasis in type II diabetes patients.

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