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Antifungal Potential Of Chlorhexidine, Honey And Propolis Against Oral Candida Albicans - An In Vitro Study

Research Article

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Abstract

Oral washes are good alternatives to lotions of chemical composition like chlorhexidine, and because of this there has been an increase in interest in natural alternatives such as honey and propolis, which have proven their effectiveness over time.
Aim: The study aimed to study the effect of oralwashes honey 50%, chlorhexidine 0.12% and propolis 5% on oral Candida albicans.
Materials and Methods: The sample included 60 syrian children (30 males - 30 females), between (6-12) years This study continued from September 2020 until February 2021, a saliva smear was taken for each child Before oral washing with research materials and swab after oral rinsing using 10 ml of solution for 30 seconds, then cultivating it in the laboratory where it is grown on SabouraudActidione Agar and conducting a count of the results and comparing the averages before and after and the effect of each substance on the oral oral Candida albicans.
Results: after one minute of using the mouthwashes the rate of decrease in Candida albicans bacterial colony count was statistically significant in the chlorhexidine mouthwash as it was 85.27%, in the propolis mouthwash it was 90.43% , and in the honey mouthwash it was 81.00% .
Conclusion: This study demonstrated the efficacy of oral chlorhexidine, propolis and honey mouthwashes on oral oral Candida albicans where the largest decrease effect was in propolis, then chlorhexidine, then honey, This indicates that the three substances are effective in reducing oral Candida albicans, which is why it is recommended to use them when needed to reduce oral Candida albicans.

Keywords: Mouthwashes; Candida Albicans; Honey; Propolis; Chlorhexidine.

Introduction

Candida is the main causative agent of Candidiasis, especially Candida albicans, and most studies have indicated that Candida albicans comprises more than (60%) of the isolated species from Candida infections [7].

Budtz-Jorgensen and Lombardi [9] noted that the adhesion of this Candida to Mucosa has an effective effect in causing injury and this is considered a preliminary step, and it seems that there is a close relationship between the adhesion of different types of Candida and their ability to cause infection.

Candida in humans is due to induction of hypersensitivity, or due to the secretion of potent toxins [9].

Sato et al 1997 reported that Candida albicans is an important factor in causing Denture stomatitis [28].

It is noteworthy that the infection caused by these types of bacteria is increasing in various parts of the world. Ashman and et al 1999 stated that Candida albicans are present in coexistence in our human societies, yet they are considered an opportunistic pathogen to cause fungal infections in the oral mucous membrane (Oral thrush) [3].

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Oral candida is one of the most common types of oral flora found in the form of flora Oral normal in 50% of healthy people(8).

For a long time, chlorhexidine has been the most widely used oral washes for reducing plaque and gingivitis, and there is no difference between an alcohol-based or a water-based lotion, and it is considered the gold standard among oral washes [11].

Chlorhexidine was developed over 50 years ago and first appeared in Imperial Laboratories in Britain in 1940 and was marketed in 1953 as an antiseptic ointment for skin wounds [16]. It was also used previously in treating skin, eye and throat infections in humans [4].

Several studies have proven that it is Bactericidal in high concentrations and Bacteriostatic in low concentrations [21].

Chlorhexidine has been shown to have the greatest immediate effect on bacterial elimination, mainly in vivo as compared to other oral antiseptics [5].

It used in patients with mental and physical disabilities to improve gingival health [27], And in immunosuppressed patients exposed to oral infections, especially Candida albicans [4].

Chlorhexidine is considered a broad-spectrum antibacterial against Gram-positive and Gram-negative bacteria, fungi, and some viruses, and it has an active effect against Streptococcus mutans [15].

Honey is one of the most used substances in complementary medicine, because it has a sweet taste and in recent times it has been used in medicines or as pharmaceutical alternatives and has attracted the interest of scientists and researchers at all levels [24]. It is believed that honey can be used as a topical wound treatment [25].

In a review presented by Yaghoobi and his colleagues, they concluded that honey contributed to wound healing because it is considered an anti-bacterial, anti-inflammatory, anti-viral, and antioxidant [33].

And many studies have determined that honey has anti-fungal properties [22], Honey not only prevents the fungus from growing, it also reduces the production of mycotoxins [26].

Canonico has found that honey can be an alternative to the antifungal medication in treating Candida albicans. Honey's antifungal activity depends on changes in the cell life cycle, cell membrane, mitochondrial function, and DNA [12].

Propolis is a fixative and supportive gummy resin for filling holes and openings in the Beehive, which bees collect from tree buds [1].

Propolis is a mixture of resin gum materials with a dark brown or greenish color and a fragrant smell, collected by bee from trees and pollen grains for some plants and kneaded with wax and add some salivary and waxy secretions to it, then the substance turns into propolis [23].

Propolis is mainly a bacteriostatic inhibitor and bactericidal in high concentrations, and its effectiveness is against Gram-positive and Gram-negative bacteria [10].

Where propolis was used in most of the research related to the maintenance of oral health, either in the form of alcoholic extract, water-based oral mouthwashes, or in toothpastes [32].

Barrientos et al., 2013 confirmed the promising efficacy of propolis in the prevention of dental caries and some diseases of the oral cavity, when they demonstrated the bio-efficacy of the ethanoli extract and metanoli of the Chilean propolis samples against the bacteria that cause caries , Streptococcus sobrinus and Streptococcus mutans [6].

Capistrano et al. (2013) found that Brazilian green propolis has a similar effect to miconazole in the treatment of Candida albicans associated with stomatitis caused by Removable denture [13].

Materials and Methods

Aim of study : Study of the effect of mouthwashes chlorhexidine 0.12% - honey 50% - propolis 5% on oral Candida albicansin children.

The study included 60 children (30 males - 30 females) who had good oral health and did not suffer from: gingivitis , wear fixed or movable braces, and take antibiotics.

Inform consent was taken from the volunteers before starting the study according to a special form designed for the research that includes details of the research and the materials used in the research.

We used local products that are widespread in pharmacies. The children were divided into 3 groups, each group containing 20 children (20 chlorhexidine - 20 honey - 20 propolis).

Saliva collection mechanism: (19)

The child was brought to the clinic and the guardian requested the following:

1. Avoid food with a high content of acids and sugars 60 minutes before work in order to cause it to reduce the pH of saliva, which leads to an increase in bacterial growth.
2. Avoid foods and drinks containing caffeine for 12 hours before work.
3. That the sample be collected between 9 - 12 p.m.
4. Emphasis on not brushing the teeth on the same day in order to preserve the oral flora and not to have bleeding that affects the accuracy of reading the results.
5. Do not use any oral rinse or paste containing chlorhexidine 7 days before the start of the clinical procedures.

A first saliva sample was taken before using rinsing by using a sterile salivary swab for this procedure. The swab included passing the swab head over the vestibular surfaces of the teeth, the palate, the floor of the mouth, and the vestibule of the cheek. Then , the child was asked to rinse his mouth with solution using 10 ml of solution for a period of time [30] seconds, After that, we took a

second swab for the child, similar to its procedures for the first one, and the child's data were recorded on it (name - age - gender - swab before/after) on each cotton swab and sent to the bacterial culture laboratory at Hama National Hospital - Hama city - syria, to start the laboratory work procedures.

• Chlorhexidine sample (20 children)
The product used is a 0.12% chlorhexidine solution ready for use, produced by a national company.
• Propolis sample (20 children)
The product used is a 5% propolis solution ready for use, produced by a national company
• Honey sample (20 children)
The honey was 100% concentrated from the packing of a national company. We drew 5 ml of honey and added it to 5 ml of distilled water in a sterile sample collection package, and we mixed it and then gave it to the child to rinse with it.

Procedures for bacterial culture in the laboratory:

Sampling extension:Wedilate the saliva samples in two stages to reduce the bacterial load for ease of counting, provided that the real concentration of germs is calculated later as follows:

Stage one: Use of 10000 microliter (10 ml) of salin by disposable one use syringe and put it in the glass tube. Then , we remove 100 micron. Thus, we get 9900 microliter in the tube. Then we add 100 micron of the saliva sample by micropipett. Finally we get dilate of saliva sample in 1/100 radio; 10-2. Then we mixed the homogenous dilate saliva sample on viberator for 30 seconds .

Stage two: Repeate the previous stages but by adding 100 microliter of dilate solution to 9900 microliter from the saline in the other glass tube and the ratio became 1/10000 and then repeate this homogenoue process by viberator.

Bacterial culture:
Culture medium: Sabouraud Actidione Agar
Culture method: we take 10 microliter of dilate solution by micropipette. And then we spread it on the surface of culture medium in Petri dish by sterilize platin loop tool in way that we get a distinct bacterial colony. And placed upside down within the incubator At 27 ° C for 48.

Bacterial appearance: The colonies appear white, flat or domed, increasing their surface over time.

Colonies counting: we count the colonies by using An Electron microscope, After the colonies counting is complete. This number presents the number of colonies in 10 dilate microliter .Thus, we multiply this number with 10000 which is the dilation ratio then we divide the number by 1000 to get the final number of bacterial colonies in ml (CFU/ml).

Statistical analysis: The data were analyzed using the statistical analysis program SPSS, version 13.00, at a confidence level of 95% (P <0.05 (We used Paired sample T test to compare the variable means of the values of the studied variables.

Results

Tables 1-3.

As for Figure No. (2), it shows the percentage values of the amount of decrease in number of Candida albicans between the two times (before using the substance and after using the sub-

Figure 1. Mean of the number of Candida albicans in the research patients.

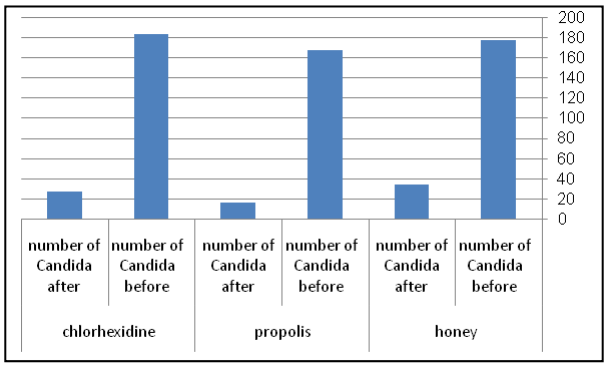

Figure 2. The percentages of the amount of decrease in number of Candida albicans between the two times (before using the substance and after using the substance) between the three experimental groups.

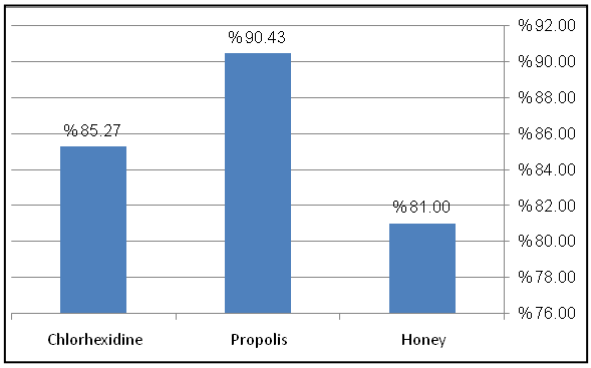

Table 1. Statistical measures of the number of Candida albicans in the research patients.

Material	Sample	Studied variables	mean	SD	minimal value	max value
honey	20	number of Candida albicansbefore using honey	177.4	57.68	101	299
		number of Candida albicansafter using honey	33.7	11.89	18	59
propolis	20	number of Candida albicansbefore using propolis	167.25	53.53	102	289
		number of Candida albicans after using propolis	16	5.02	10	27
chlorhex-idine	20	number of Candida albicansbefore using chlorhexidine	182.95	55.23	99	258
		number of Candida albicans after using chlorhexidine	26.95	8.23	14	38

Table 2. Results of using Paired Samples T Test when comparing the arithmetic means of the number of Candida albicans before and after the experiment.

Materials	Comparisons		difference between arithmetic means	T Test value	df	P-value	description
honey	Pair 1	number of Candida albicansbefore using honey - number of Candida albicansafter using honey	143.7	13.674	19	0	There are statistically significant differences
propolis	Pair 1	number of Candida albicans before using propolis - number of Candida albicansafter using propolis	151.25	13.935	19	0	There are statistically significant differences
chlorhex-idine	Pair 1	number of Candida albicans before using chlorhexidine - number of Candida albicans after using chlorhexidine	156	14.838	19	0	There are statistically significant differences

* statistical significant p<0.05

Table 3. The percentages of the amount of decrease in number of Candida albicansbetween the two times (before using the substance and after using the substance) between the three experimental groups.

materials	number of patients	Candida albicansvariable value before using the material	Candida albicans-variable value after using the material	The amount of decrease	Reduction ratio %
Honey	20	177.4	33.7	144	81.00%
Propolis	20	167.25	16	151	90.43%
Chlorhexidine	20	182.95	26.95	156	85.27%
Total	60	175.87	54.951	121	68.75%

stance) between the three experimental groups.

Discussion

Over the last few years,, the search for new antifungal drugs has increased due to lack of effectiveness, side effects, and interactions with other drugs taken by patients. In addition, as the lesions continue to recur, the antifungal drugs used seem to be resistant to pathogens. This fact has prompted people to seek a new antifungal agent based on natural compounds such as honey and propolis [34].

Candida albicans has a excessive acid tolerance and is able to generating acids even under low pH conditions. that may favor the fungus in the microbial shifts associated with Tooth decay [14], so We did this study because the presence of *C. albicans* has been increasingly related to dental caries.

Julie's et al 2006. they was found that honey cannot be used in vivo on a large scale, as they was found that honey is limited to topical treatments, and it cannot be used to treat candidaemia, which is the most dangerous form of candidiasis [20].

In Sayyadi et al., 2020 study, they compared the ethanolic and aqueous extract of Iranian propolis against oral candidiasis isolated from oral in chemotherapy treated patients. The aqueous and ethanolic extracts of Iranian propolis showed antifungal activity against each of *C. albicans* isolated from patients, but the ethanolic extract was more effective than the aqueous extract [29].

In Christian's study, the aims of this study were to evaluate the antifungal activity of six commercial propolis extracts against Candida have been isolated from the oral cavity from patients have Removable dentures, The results in this study show that all the propolis extracts that were evaluated are able to inhibit Candida development [18].

In the Freires study, to evalute the antifungal activity of two varieties of Brazilian propolis, it was found that Brazilian propolis has strong activity against Candida and should be considered promising candidates for the treatment of oral and systemic candidiasis [17].

In Shrestha al 2011 study, mouthwashes containing chlorhexidine were able to kill all strains of Candida albicans and Candida tropicalis in shorter times compared to mouthwashes containing thymol [30].

We disagreed with the Talebi et al 2014 study because this study showed that the chemical mouthwash had a better effect than the herbal mouthwash [31].

In Aroonrerk's et al 2007 study of 6 commercial mouthwashes containing fluoride (FLO), cethylpyridinium chloride (CPC), chlorhexidinegluconate (CHX), triclosan (TRI) and herbal extracts: Twin Lotus (TLO) and Herbric concentrated (HBC) ,They found that CHX and triclosan mouthwashes were effective in reducing oralCandida activities, This differs with our study where natural mouthwashes (propolis) was better than CHX in eliminating Candida albicans [2].

We disagreed with Where it was concluded that propolis exhibited weaker antifungal activity against oral fungi while only honey produced from Trigona spp. Had strong antifungal activity compared to other honey against the fungi of mouth involved in denture stomatitis [34].

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