Evaluating the Antifungal Efficacy of Incorporating Kappa-Carrageenan Powder Into "Heat-Cured, Acrylic-Based Soft Denture Lining Material

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Abstract

Denture stomatitis is one of the serious problems that are related to the continuous use of soft lining material due to the accumulation of microorganisms. It is caused mainly by fungal growth, especially of *Candida albicans*. It, therefore, becomes necessary to examine the effectiveness of incorporating an antifungal drug into the soft lining material.

This study was conducted to investigate the efficacy of adding the kappa-carrageenan powder to heat-cured, acrylic-based soft lining material against *Candida albicans* adherence.

A pilot study was performed to decide the best concentration of kappa-carrageenan to be used with the heat-cured, acrylic-based soft liner. Five percentages by weight of kappa-carrageenan powder (0.5, 1, 1.5, 2, and 2.5wt.%) were evaluated and compared with the control group (0wt.%). The *Candida albicans* adherence test was performed to assess the efficacy of kappa-carrageenan powder in preventing the adherence of *Candida albicans.* Results showed the 1.5wt.% and 2wt.% groups as having the best effect.

The main study included preparing thirty samples and dividing them into three groups based on the percentage of powder of kappa-carrageenan added to the heat-cured acrylic-based soft lining material (control: 0wt.%; experimental: 1.5wt.% and 2wt.%). The purpose of performing *Candida albicans* adherence test was to evaluate the antifungal efficacy of kappa-carrageenan powder addition, and all the resulted data were analysed using "one-way analysis of variance (ANOVA) and Dunnet T3 post-hoc test at a significance level of $p<0.05$".

The results of *Candida albicans* adherence test revealed a highly significant decrease in the values of the adhered *Candida albicans* cells after incorporation of 1.5wt.% and 2wt.% of kappa-carrageenan powder when compared to the control group ($p<0.01$).

The result of this study suggests that the addition of kappa-carrageenan powder is an effective drug against *Candida albicans* adherence on the surface of the heat-cured acrylic-based soft lining material, and the addition of 2wt.% is more effective than 1.5wt.%.

Keywords: *soft liner, kappa-carrageenan, Candida albicans, denture stomatitis.*

Introduction

Denture resilient lining materials that are applied over the intaglio surface of the denture to work as a cushion to absorb the stress generated by occlusal forces and to decrease continuous trauma on the denture-bearing area, making wearing the denture more tolerable to the patients[(1)].

The ideal soft lining material exhibits a number of properties to ensure maximum benefits for denture wearers; which may include dimensional stability, colour stability, good resiliency, biocompatibility, sufficient bond strength with the denture base material, low water solubility, and resistance to microbial growth[(2)].

The colonization of microbes in the soft lining material is one of the serious problems that affect its long-term efficacy. The most common clinical condition associated with this problem is "denture-induced stomatitis"; this condition is mainly caused by *Candida albicans*, which is the most popular fungi responsible for oral infections[3].

Topical antifungal medicaments can be considered as the most common line of treatment for "denture-induced stomatitis". However, certain difficulties are associated with this type of management, such as the inability of the elderly to deliver the optimum dosage of the drug due to a lack of motor dexterity. To overcome this issue, a suggestion has been made to develop a drug delivery system by incorporating the drug into the denture materials. Furthermore, the development of fungal resistance against the drug due to its continual use for a prolonged time makes it necessary to search for a new effective medicament to be used as an alternative to synthetic drugs[3,4].

Herbal medicines have proved to be an excellent alternative treatment line for the management of oral infections, and this makes it necessary to investigate these products to ensure their biological safety and antifungal properties[5].

Carrageenan is a water-soluble sulphated galactan and major cell-wall component in red algae. It has been identified as possessing high anti-coagulant, anti-oxidant, anti-tumour, and anti-microbial activity[6]**.** In addition, carrageenan has been used for many years as a food additive and in pharmaceutical applications due to its stabilizing, thickening, and emulsifying properties, and it is accepted by the U.S. Food and Drug Administration(FDA) and the World Health Organization(WHO). The recommended usage of carrageenan as an additive is advised to be in the range of 0.005–3%[7]. Its suggested that these sulphated polysaccharide are negatively charged molecule which have inhibitory effect through its ability to produce alteration in the cell wall by interacting with the positive charge on the cell surface[8]. Thus, this study was conducted to evaluate the effect of kappa-carrageenan against *Candida albicans* adherence to the heat-cured, acrylic-based soft lining material.

Materials and Method

Pilot study

Heat-cured, acrylic-based soft liner(Vertex,Netherlands) was used. Kappa-carrageenan powder(Sigma-Aldrich,Denmark) was added to the powder of the soft liner in six percentages by weight of the powder (0, 0.5, 1, 1.5, 2, and 2.5wt.%). These groups were used to decide the two groups that produced the best effect against *Candida albicans* adherence. Mixing was done according to manufacturer instructions for the control group without the additive (0wt.%), while for the experimental group, the weight of kappa-carrageenan powder was subtracted from the weight of the soft liner powder to maintain an optimum powder/liquid ratio[9]. The *Candida albicans* adherence test was conducted; four samples were used for each group. The results of the pilot study demonstrated that 1.5wt.% and 2wt.% produced the best effect against *Candida albicans* adherence to the denture soft lining material, so it was selected for the main study.

The pilot study also assessed the efficacy of using kappa-carrageenan powder in disinfectant form by adding it to the distilled water to be used as an immersion solution for denture disinfection. However, as the powder was added to the distilled water in the room temperature, it formed a gelatin that was thick in consistency; thus, the procedure was excluded from the main study due to a lack of feasibility.

The main study

A total of thirty samples were prepared and divided into three groups, with ten samples for each group: control group(C):heat-cured, acrylic-based soft liner without the additive; experimental group(E1):heat-cured, acrylic-based soft liner with 1.5wt.% kappa-carrageenan powder additive; and experimental group(E2):heat-cured, acrylic-based soft liner with 2wt.% kappa-carrageenan powder additive.

Candida albicans adherence test

Sample preparation

A plastic disc measuring 10×2 mm in diameter and thickness, respectively, was fabricated to make the final shape of the soft liner samples used for the *Candida albicans* adherence test[10]. These plastic molds were invested in addition-type silicone material (Zermack,Italy); molds with the silicone replica were

then invested in the lower portion of the dental flask with freshly mixed dental stone (Zermack,Italy). Samples of the control group were prepared according to manufacturer instructions for the heat-cured, acrylic-based soft liner (powder/liquid ratio:1.2g powder/1ml monomer), and mixed together using a clean glass container. For experimental samples, the weight of the kappa-carrageenan powder was subtracted from the weight of the soft liner powder to obtain an accurate powder/liquid ratio. An amalgamator device (Perfection Plus,United Kingdom) is used to mix the two powders for 40 seconds to obtain a homogeneous mixture. All samples were cured according to manufacturer instructions using a thermostatically controlled water bath (Lab.tech.,korea) heated to 70□ for 90minutes; temperature was then raised to 100□ for 30minutes. After processing, samples were finished with sharp scissors and polished with a fine-grit, silicone polishing bur under continuous water cooling. All samples were stored in distilled water for 24hours at 37□ before being tested to eliminate any residual monomer[11].

Isolation and identification of *Candida albicans*

A patient with signs and symptoms of "denture-induced stomatitis" attending the College of Dentistry/ University of Baghdad was selected . The *Candida* was isolated from the patient's mouth using a sterile cotton swab and gentle rubbing of the intra-oral lesion[12], which was cultured on the surface of a "Sabouraud dextrose agar"(SDA;Oxoid,England) plates and incubated at (37°C) for 48hrs.

Candida albicans identification was made using macroscopic examination. The *Candida albicans* had a pearl-shaped appearance, with a creamy and pasty texture on SDA[13]. A microscopic examination was conducted using a light microscope(Olympus,Japan) and gram stain procedure(Fig.1)[14] biochemical identification was made using the API-20C-AUX system and API-*Candida* system [15].

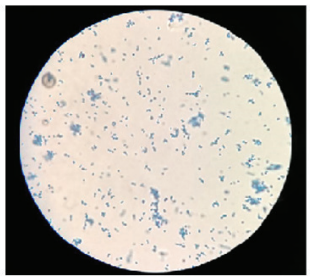

Figure 1: Microscopic examination *Candida albicans* on sabouraud dextrose agar (SDA).

***Candida albicans* adherence test**

The *Candida albicans* adherence test was accomplished by preparing a *Candida albican*s suspension using normal saline, which is equivalent to 0.5 McFarland standards. Then, 0.1% of *Candida albican*s suspension was added to a test tube containing 0.9% sabouraud dextrose broth(Oxoid,England) using micropipette; samples were immersed in this tube and incubated at (37°C) for 1hr. After incubation, samples were taken out of the suspension and washed with phosphate-buffered saline for 1minute, and dried using absorbent paper. Fixation of the adherent cells is done using methanol 80% for 30seconds, followed by staining with crystal violet stain for 1minute[16].

An inverted light microscope(Karl Kolb,Germany) was used to examine the samples using 40×magnification power, with the microscope being connected to a computer. Four standardised fields were examined in each sample[17].

Statistical analysis

Results of the presented research were analysed using SPSS "version 24 computer software". The descriptive statistics that have been "made, includes means, standard deviation, and graphical presentation by bar chart". "Inferential statistics" were also made, including one-way analysis of variance (ANOVA), for comparison means among all groups, and Dunnet T3 multiple comparison tests, which shows the significance between each of the two different groups, with $P<0.05$ considered significant.

Results:

Evaluating *Candida albicans* adherence

Results of the *Candida albicans* adherence test were evaluated by counting the number of adhered *Candida* on the surface of the control and experimental samples through the use of an inverted light microscope, in which it appeared as round or oval violet cells(Fig.2). The results for both experimental groups showed lower mean values compared with the control group, with the experimental group(E2) revealing the lowest mean value of *Candida albicans* (4.910 cells) compared with the control group(C), which had the highest values of adherent cells (36.600 cells) (Fig.3; Table1).

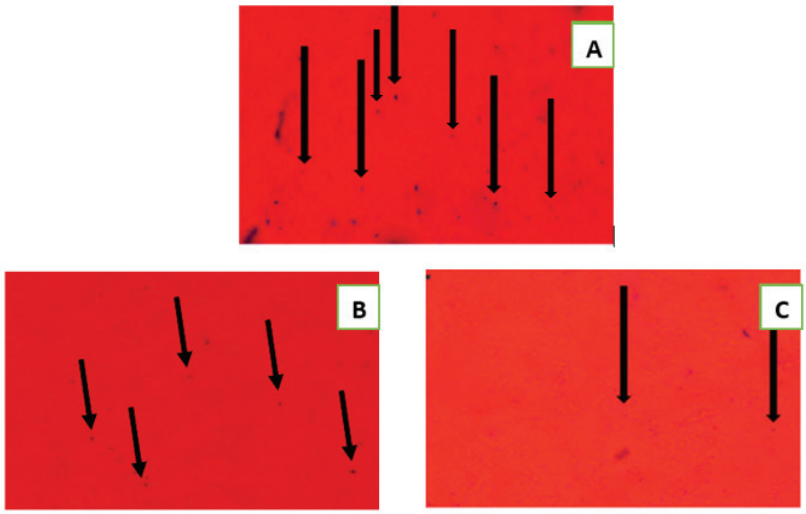

Figure 2: Microscopical image of *candida albicans* on soft liner samples. C) Control samples (without additive), E1) expiremenatl samples (with 1.5 wt.% of kappa-carrageenan additive), and E2) expiremental samples (with 2 wt.% of kappa-carrageenan powder additive).

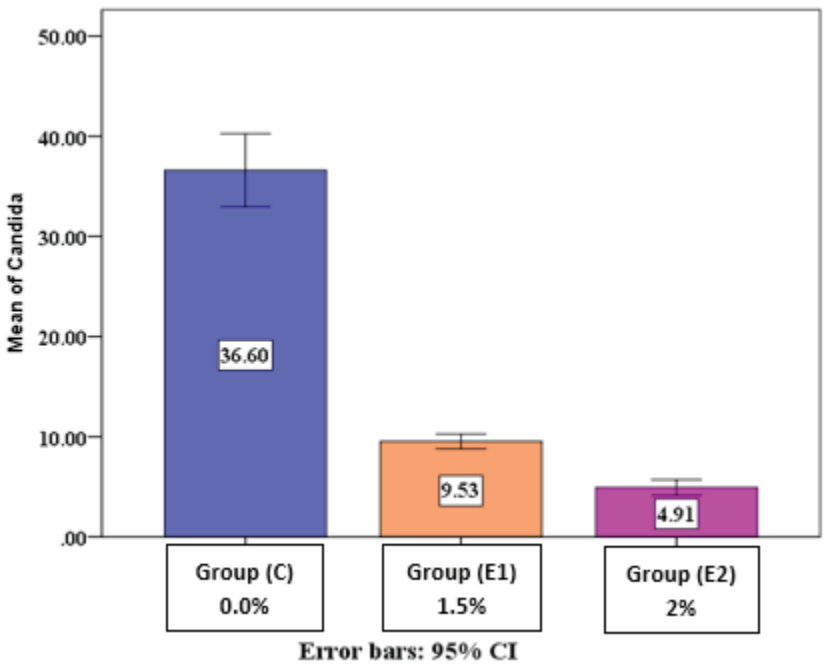

Figure (3): Bar chart showing mean values and standard deviation of *Candida albicans* adherence for control (C) and experimental groups (E1 and E2).

Table (1) Descriptive statistics of *Candida albicans* adherence test.

Groups	Mean	SD	SE	Minimum	Maximum
C	36.600	5.119	1.619	30.000	44.300
E1	9.530	1.027	.325	8.000	11.300
E2	4.910	1.101	.348	3.300	7.000

Results of the one-way ANOVA test were highly significant among all groups (p<0.01). Dunnet T3 test showed a highly significant decrease for both experimental groups compared with the control group, and there was also a highly significant difference between the two experimental groups (p<0.01); Tables2 and 3.

Table 2: Statistical test of *Candida albicans* adherence among groups using one-way ANOVA

	Sum of Squares	df	Mean Square	F	Sig.	Effect size
Between Groups	5861.285	2	2930.642	308.814	.000 [HS]	0.958
Within Groups	256.230	27	9.490			
Total	6117.515	29				

Table (3): Multiple comparisons of *Candida albicans* adherence between groups using the Dunnett T3 post hoc test.

(I) Groups	(J) Groups	Mean Difference (I-J)	Sig.	
C	E1	27.070	.000	HS
	E2	31.690	.000	
E1	E2	4.620	.000	

Discussion

Herbal medicine is considered to be very reliable alternative to antimicrobial drugs, with little or no side effects; for this reason, there is worldwide interest in medicinal plant extracts. Therefore, much research has been conducted regarding their biological safety [(4)].

Kappa-carrageenan is a sulphated polysaccharide and major cell-wall component in red algae. Carrageenan has been studied recently for its several medicinal benefits, and it has been suggested that it can alter the cell wall by interacting with the positive charges on the cell surface [(8)].

The *Candida albicans* adherence test was performed because the adherence of *Candida albicans* on the fitting surface of dentures has been proven to be of critical importance in the development and maintenance of denture stomatitis in continuous denture wearing [(18)].

In this study, the incorporation of kappa-carrageenan into the heat-cured, acrylic-based soft lining material resulted in a decrease in the number of *Candida* cells adhered to the surface of the experimental samples when compared to the control groups.

The effect of kappa-carrageenan on *Candida albicans* is explained by Souza et al. (2018) who stated that these macromolecules possess antifungal activity and induce resistance to fungal organisms. This is in agreement with Soares *et al*. (2016) who suggested that carrageenan extracts promote morphological alterations in the cell wall of *Candida* species. After the exposure of *Candida* cells to kappa-carrageenan, the chitin cell wall content is decreased significantly. This alteration is associated with a decrease in beta-glucan content, which may influence cell death.

Another factor that affects the adherence of *Candida albicans* cells is that the surface of the soft lining material is considered to be a hydrophobic surface that enhances the adherence of the *Candida* species. The use of kappa-carrageenan, which is considered to be a hydrophilic material, may improve the hydrophilicity of the surface of the soft lining material, which eventually minimises the adherence of *Candida albicans* on the surface of

the heat-cured, acrylic-based soft lining material, as suggested by Yoshijima in 2010 [21].

The highly significant difference that occurred between the two experimental groups (E1 and E2) is due to the increased concentration of kappa-carrageenan powder, which resulted in an increase in its effect [22].

Conclusion

From the presented research, it can be concluded that kappa-carrageenan powder can be considered as powerful antifungal material and the incorporation of kappa-carrageenan powder into "heat-cure acrylic-base soft lining material" can successfully be accomplished to produce a soft lining material with antifungal properties against *Candida albican*s microorganisms. Also, experimental group(E2) showed a better antifungal activity when compared to the control and experimental group(E1).

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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