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THE EFFECT OF NYSTATIN AND FLUCONOZALE (ANTIFUNGAL AGENTS) ON HARDNESS AND IMPACT STRENGTH OF HEAT –CURE ACRYLIC RESIN

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Abstract

When denture is worn in the mouth for considerable period of time, it will become colonized with microorganisms and causing denture stomatitis, it is commonly found in the maxillary arch, rarely in the mandibular arch, and it is not uncommon under removable partial denture base in the maxillary arch. Denture trauma, allergy, poor oral hygiene, PH level of saliva, age, sex, smoking and immune system deficiency are generally regarded as etiological factors in denture stomatitis .The significant causes of denture stomatitis are trauma and infection with Candida species .The most important predisposing factor for Candida induced denture stomatitis is the presence of denture or any appliances in the oral cavity, indirectly, this has been proved since removal of the denture will cause the infection to disappear. Prosthetic treatments include removal of dentures, lining of dentures, making new denture and disinfection of dentures. In this study mixed Nystatin and Fluconozale (antifungal agent) with acrylic resin and evaluated the effect of these materials on some mechanical properties (hardness strength & impact strength).

This study was clearly shown that statistically non significant differences in shore hardness strength of Fluconozale group and Nystatin group, and highly significant difference in impact strength of Fluconozale group and Nystatin group when compared with control groups .This mean the Fluconozale & Nystatin less effect on hardness strength, but more effect on impact strength.

ألخلاصه //

أن أستخدام طقم الأسنان لفترة من الزمن يصبح مستعمرة لتجمع الجراثيم مما يؤدي الى ألتهاب الفم, وغالبا" الألتهاب ما يكون في الفك الاعلى ونادرا" في الفك الاسفل , واقل شيوعا" في حالة الطقم المتحرك في الفك الاعلى . الجرح من الطقم ,والحساسيه , سوء الحاله الصحية للفم , معدل PH في اللعاب , العمر,الجنس , والتدخين , نقص نظام المناعه , تعتبر من العوامل المهمه لألتهاب الفم. وأن من أهم الاسباب المؤديه لهذه الألتهاب هي الجرح الناتج من الطقم السني او الأصابة بمرض الفطريات, وغالبا" مايختفي عند رفع او ازاله الطقم من الفم . أن ازاله الطقم او تبطين الطقم السني تعتبر من أهم الطرق لمعالجة ألتهاب الفم وكذلك عن طريق استخدام المضادات الحيويه للفطريات لغرض السيطره على مرض الفطريات . في هذه الدراسه تم خلط مادة (النستاتين والفلوكونوزول) مع الأكريلك وقيمت تأثيرات تلك المواد على بعض الصفات الميكانيه (الصلابه , قوة الصدمه).

اظهرت النتائج بشكل واضح عدم تأثير هذه المادة على قوة الصلابه , بينما أثرت بشكل كبير على قوة الصدمه .

Introduction

The method of using acrylic resin in the form of liquid monomer and powder polymer was introduced in 1937 and was know as the "dough method" most of the present day acrylic supplied in this form [1, 2].

Denture stomatitis is a common and recurring problem of denture wearers[3] . Conventional management of denture stomatitis consist of topical application of an oral antifungal agent [4] ,or replacing the old denture by new one or relining the old denture by cold cure acrylic or tissue conditioner then make occlusal grinding to have balance occlusion and articulation.[5].

Antifungal drugs to control Candida infection were also important to treat denture stomatitis. Nystatin is the drug of choice because of its established effectiveness against Candida and other yeasts and because of the lack of side effects .Nystatin must be used locally since it is not absorbed from the gut. According to [6] the available preparation:-

1- Nystatin oral suspension: 100,000 I.U/ml which can be applied to the fitting surface of denture four times during the day.
2- Nystatin oral tablets: each tablet contains 500,000 I.U. which can be sucked three times a days with dentures out side the mouth.
3- Nystatin ointment: it contains 100,000 I.U/ gm.

Also fluconozale is a systemic antifungal agent, has been shown to be effective in the treatment of oropharyngeal candidacies, [7]. Many studies mentioned the effete of antifungal agents on mechanical and physical properties of denture base materials and reline materials ,such as [8]concluded that the most effective does of nystatin mixed with heat cured acrylic resin disc measuring 2mm thickness and 50 mm diameter was 1500,000 I.U that give 100% inhibition of candidal growth for 24 days , and [9]studied the effect of antifungal agent incorporated with heat processed (poly methyl methacrylate) denture base material as a relined materials on the physical and mechanical properties of acrylic denture base materials ,the result revealed highly significant decreasing on (transverse strength ,modulus of elasticity ,impact strength ,tensile bond strength and indention hardness test ,and there is significant increase in water sorpotion and solubility test. Also[10] studied the effect of miconazole incorporated into different reline materials on (tensile , shear bond strength ,water sorption ,solubility and surface roughness),the results showed that there is reduction in bond strength (tensile and bond strength) , and the results of sorption test shown an increases in the mean value for each reline materials is observed after incorporation of miconazole and reduction in solubility value of each reline materials ,but there is non- significant increase in surface roughness of reline materials after miconazole incorporated . Hardness has often been used as an index of the ability of material to resist abrasion or wear. However ,abrasion is a complex mechanism in the oral environment ,for this reason the reliability of hardness as a predictor of abrasion resistance is limited .The fracture of dentures may be due to mechanical properties of the acrylic resin or may be due to a multiplicity of factors leading to failure of denture base material ; these factors include composition of the resin ,processing technique ,and conditions presented by the oral environment [11,12].Addition factors which form areas of stress concentration such as large freak notch ,denture with thin or under extend flanges ,poorly fitting dentures or lack of adequate relief ,denture with wedged or locked occlusion ,poor clinical design and dentures which have been previously repaired[13]. The present study was carried out to evaluate the effect of addition of two types of antifungal agent(Fluconozale & Nystatin) on some mechanical properties (impact strength and hardness strength) of pink heat cure acrylic resin.

Materials & Methods

* Some materials & equipment used in this study:

- Pink heat cure –acrylic resin (Turkey).
- Antifungal agents include:
  1- Nystatin B.P… 500,000 I.U (Asia COM., Syria).
  2- Fluconozale capsule (CIPLA LTD. India).
- Dental stone (Q.D England).
- Separating medium (Dentaurum, Germany).
- Distilled water (Baghdad Company, Iraq).
- Water bath (Bego, Germany).
- Vibrator (wipla Germany).
- Bench press (Germany).
- Digital sensitive balance (A & B COM., Japan).

Grouping the specimens

Sixty sample were prepared and divided into two categories(30 samples in each categories) depended on test (shore hardness test & impact strength) ,each categories contain three groups (G1,G2,and G3) .The samples include :

Group 1: Control group (untreated group).
Group 2: Acrylic denture base mixed with 500,000 I.U Nystatin.
Group 3: Denture base acrylic incorporated with 150mg Fluconozale.

General preparation of the acrylic specimens

Instead of wax pattern preparation which needs more time and effort in its preparation and wax elimination procedure, the metal pattern was constructed with a dimension of (55mm x10mm x2.5mm) length, width, & thickness respectively used for hardness test & impact test.

The conventional flasking technique for complete dentures was followed in the mold preparation. Two metal patterns were coated with a separating medium and allowed to dry before investing them in the lower half of the flask which contained stone mixed according to the manufacture instruction, and allowed to set .The stone and metal pattern were coated with separating medium and allowed to dry and then the upper half of the flask was assembled and filled with stone mixtures and allowed to be harden for (60) minutes before the flask was opened .After removal of the metal patterns carefully with help of the wax knife, the two halves of the mold were coated with separating medium to be ready for packing with acrylic dough.

Pink heat –cure acrylic powder with liquid was mixed according to manufactures instructions, the liquid was placed in a clean and dry mixing vessel followed by slow addition of powder .The mixture was then stirred with wax knife and left to stand in a closed container at room temperature until reaching the dough stage .For packing into gypsum mould when it was separated cleanly from the walls of the mixing jar .The acrylic resin dough was used when no more stuck to the vessel walls, the resin was removed from its mixing container and rolled ,then packed into the mould which had been treated with separating medium .The same procedure was repeated for groups (2) and groups(3), but before the acrylic material was mixed antifungal agent were allotted by weight using sensitive balance .The antifungal powder was incorporated uniformly into the denture base powder by spatulation .The amount of liquid was increased by a bout 0.75cc for the does 500.000I.U and about 0.2 cc for doses 150mg Fluconozale , in order to reach the right consistency of dough [8,14,15] .The two halves of the flask were closed together and placed under the hydraulic press ,and then the pressure was slowly applied to allow even flow of the dough throughout the mould space .The pressure (20 bar) was then released. The flask was opened and the over flowed material (flash surrounding the mould space was removed with wax knife. A second trial closure was again coated with the separating medium ,allowed to dry .The two halves of the flask were finally closed until an intimate contact had been established and left under the press (1500 psi)for (5) minutes before clamping was done then the flask was placed in a flask clamp maintaining undisturbed pressure during processing[16]. Curing was carried out by placing the clamped flask in a thermostatically controlled water bath and processed by heating at (74 C) for (1.5) hour and the temperature was then increased to the boiling point for half an hour [17] .After completing the curing ,the flask was allowed to cool slowly at room temperature for (30) minutes ,followed by a complete cooling of the flask with tap water for (15) minutes before deflasking .The acrylic pattern were then removed from the stone mould . All flashes of acrylic were removed with an acrylic bur to get a smooth surface; the stone bur should be used followed by (120) grain size sand paper to remove any remaining small scratches with continues water cooling. While polishing was accomplished by using bristle brush and pumice with lathe polishing machine, a glossy surface was obtained with wool brush and polished soap on dental lathe using low speed (1500rpm and the specimens were continuously cooled with water to avoid over heating ,which may lead to distortion of the

specimens . All the tested specimens were conditioned in distilled water at 37 ^{0}C for 50 hours before they were tested according to [18].

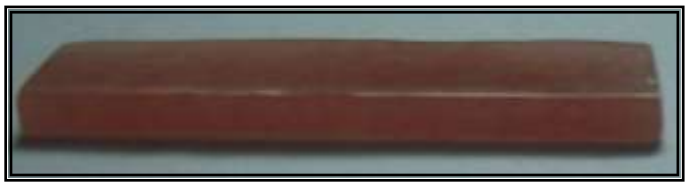

Figure (1): The final shape of each sample before test

Hardness strength (Test equipment and procedures):

Acrylic resin specimens were prepared as described previously the final dimensions were 55x 10x 2.5mm. Shore hardness tester was used in this study for measuring the indentation hardness of the specimens the test load was set to 50N for shore "D" which is suitable for acrylic resin material. The contact surface of the shore hardness tester must be parallel to the specimen support of the test stand to prevent errors in measurements. The distance between the specimen surface and the indenter of the hardness tester was set to be 5-12 mm. During carrying out the test the contact period between the specimen and the indenter was 6 second. After that the measurement were taken directly from the scale reading. Five measurements were done on different area of each specimen and the average of five reading was calculated.

Impact strength (Test equipment and procedures):

Evaluation of impact strength was done according to the procedure given by the [19] with charpy type impact machine figure (2) which was supplied with a pendulum .In this procedure ,weights differ according to the material to be tested .The specimens were held horizontally and struck by the pendulum of (2j) capacity at the center of tested specimen in the center area .The scale reading gives the impact energy in (j) .figure (3)The value of charpy impact strength was computed by the following formula according to [20] for determination of impact strength (KJ/M^2) = E/tw Where E is the absorbed energy in (KJ) ,while t is the thickness of the specimen and w is the remaining width at the notch base in (m^2) .

Figure (2): Charpy machine (Impact strength).

Figure (3): The scale reading gives the Impact energy in (j)

Statistical Analysis

The suitable statistical methods were used in order to analyze and assess the results, they include the followings:

1- Descriptive statistics:

A) Statistical tables including observed frequencies.

B) Summary statistic of the readings distribution (mean, SD, SEM, minimum & maximum).

C) Graphical presentation by (Bar –chart).

2 – Inferential statistics:

These were used to accept or reject the statistical hypotheses, they include the followings:

A) Analysis of variation ANOVA (f-test).

B) Least significant difference LSD (f-test).

Results

Results of shore hardness strength test were obtained for (30 specimens), which include the three groups each group have (10 sample).

The mean of shore hardness test, standard deviation, standard error, maximum & minimum values, and inferential statistical methods represented by analysis of variance "ANOVA" test for each group are listed in table (1)

Table (1): Descriptive statistic of control group & experimental groups (nystatin& fluconozal groups).

GROUPS	No.	Mean	Std. deviation	Std. error	Range		ANOVA test (P-value)	Sig
					Minimum	Maximum		
Control group (G1)	10	89.240	1.165	.521	87.3	90.1	.112	NS
With Fluconazol (G2)	10	87.060	1.808	.808	84.7	89.7		
With Nystatin (G3)	10	87.860	1.506	.673	86.5	89.5		
Total	30							

The source of difference is investigated by further complement analysis of data by using LSD (least significant difference) test to examine the differences between the different pairs of the three groups as shown in table (2).

Table (2): Lest significant differences (LSD) test for the shore hardness test for control group & experimental groups (nystatin& fluconozal groups).

Dependent Variable: Shore hardness test

LSD

Studied groups		LSD test P-value	Sig.
Control without any addition (G1)	With fluconozal (G2)	.042	S
	With Nystatine (G3)	.176	NS
With fluconozal (G2)	With Nystatine (G3)	.420	NS

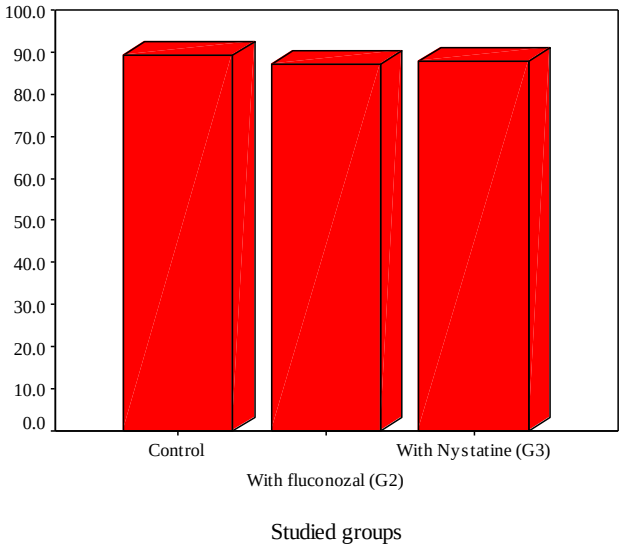

Figure (4): Histogram of shore hardness strength mean values of tested groups.

Results of impact strength test calculated in (kj/m^2) were obtained for (30 specimens), which include the three groups each group have (10 sample).

The mean of impact strength, standard deviation, standard error, maximum & minimum values, and inferential statistical methods represented by analysis of variance "ANOVA" test for each group are listed in table (3).

Table (3): Descriptive statistic of control group & experimental groups(nystatin& fluconozal groups).

GROUPS	No.	Mean	Std. deviation	Std. error	Range		ANOVA test (P-value)	Sig.
					Minimum	Maximum		
Control group (G1)	10	1.311E-02	6.0142E-04	2.6897E-04	.0125649	.0138698	.000	HS
With fluconazol (G2)	10	7.463E-03	6.9368E-04	3.1022E-04	.0066298	.0082789		
With Nystatin (G3)	10	9.580E-03	1.3921E-03	6.2257E-04	.0078627	.0109287		
Total	30							

The source of difference is investigated by further complement analysis of data by using LSD (least significant difference) test to examine the differences between the different pairs of the three groups as shown in table (4).

Table (4): Lest significant differences (LSD) test for the impact strength for control group & experimental groups (nystatin& fluconozal groups).

Dependent Variable: Impact strength test

LSD

Studied groups		LSD test	
		P-value	Sig.
Control without any addition (G1)	With fluconozal (G2)	.000	HS
	With Nystatine (G3)	.000	HS
With fluconozal (G2)	With Nystatine (G3)	.005	HS

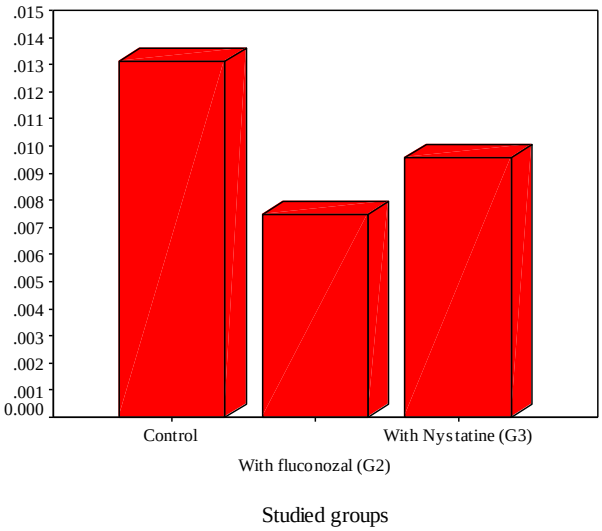

Figure (5): Histogram of impact strength mean values of tested groups (nystatin & fluconozale groups).

Discussion

Denture stomatitis is seen frequently in patients wearing ill fitting denture with unbalanced occlusion which are likely to cause trauma to the palatal mucosa, there is no convincing evidence that denture stomatitis caused by trauma as most cases clear up after treatment with fungicides there is good evidence that type I (a localized simple inflammation) is caused by trauma alone, where as, type II(a more diffuse erythema involving a part of , or the entire denture – covered mucosa) and III(a granular type (papillary hyperplasia) commonly involving the central hard palate and alveolar ridges) seen to be induced by Candida species .However, trauma may be a predisposing factor to infection [3].

The occurrence of denture stomatitis is closely related to quality and the maintenances of denture[21].

Removable appliances have the advantage of being able to be cleansed properly and allowing clearing of the stagnation areas in the mouth during the period of removal [22].

The appliance will absorb microorganism on its surface .This depend on the material used ; acrylic appliances retain a denser flora than metal probably because they are slightly porous and easily scratched ,producing areas where microbes can be retained in addition to the chemical binding to acrylic [8,14].

There are three possible alternatives for treatment of denture stomatitis:-prosthetic treatment, antimycotic therapy and surgical treatment .Prosthetic treatment include removal of dentures, lining of dentures, making new denture and disinfection of dentures .Leaving the dentures out is a possible alternative, whether the cause of inflammation is trauma or infection .It is the simplest procedure that will reduce the amount of Candida organism present in the mouth [23]. [15] found that 1000,000 I.U of Nystatin were necessary in the visco- gel liner Nystatin preparation to maintain an adequate level of antifungal activity for 2 week. And [24] found that 50 mg fluconozale daily for 14 days used for treatment of patient with denture stomatitis. Also [25] used Fluconozale capsule 50 mg daily and Itraconazole capsule 100 mg daily for treatment of denture stomatitis. While[14] found that fluconozale become in effective when incorporated into cold –cured acrylic, and heat –cured acrylic resin hard liner.

In this study ,shore "D" hardness tester was used for measuring the hardness of acrylic resin .fracture in dentures result from two different types of forces, namely flexural fatigue and impact . Impact failure usually occurs out of the mouth as a result of sadden blow to the denture or accidental dropping whilst cleaning, coughing or sneezing [12].

The majority of machines routinely used for impact testing are of two types and in both cases the blow is delivered by a weighting pendulum or tap, although the specimens are held differently:

1- A simple horizontal beams tests (charpy).

2- A cantilever beam test (Izod).

This study designed to test the some mechanical properties of acrylic mixed with fluconozale and nystatin 500,000I.U, the results mentioned in table 1, 2, 3, and 4.as the following :

In table (1) for the shore hardness test groups ,the results showed there are highly shore strength related to control group which was (89.240) ,followed by group(3) nystatin group which was (87.860) ,followed by group (2) Fluconozale group which was (87.060) .

In table (2) mentioned the LSD (least significant difference) between group (1), group (2),and group (3).

showed significant between group (1) compared with group (2) , but non significant between group (1)&group(3).Also there are no significant difference between group (2)& group(3) .According this study and results that obtained can be used the antifungal agent without effect on shore hardness test .On the other hand in table (3) for the impact test groups the results showed there are highly impact strength related to control group (untreated group) which was (1.311E-02) ,followed by group (3) nystatin group which was (9.580E-03) ,and followed by group (2)

Fluconozale group which was (7.463E-03). In table (4)mentioned LSD (least significant difference)between the difference pairs of three groups ,showed highly significant difference between group(1) & group (2) and highly significant difference between group (1) & group (3).Also highly significant difference between group (2) and group (3) .According this study and results that obtained the Fluconozale material more effect on impact strength ,but the nystatin less effect from Fluconozale on impact strength when compared with untreated group (control group) .this may be due to presence of impurities that shown when we mixed the powder of nystatin and Fluconozale with the polymer of the acrylic ,may be the causes in lowering the impact strength that give indication to the brittleness of the material. The possible explanation of these result is the mixing of nystatin and Fluconozale with the polymer of the acrylic resin so this impurity might leak out as soluble materials so increasing solubility within increasing time leaving other empty spaces or gaps for the water to get in, and within as increasing long time of storage, the material becomes stiff .The other possible explanation is the surface defect which has been found to significantly reduce the impact strength of acrylic resin [26].

Conclusion

On the basis of the results arrived at, the following conclusions can be drawn:

1- The effect of antifungal agent (nystatin & Fluconozale) on the shore hardness test values is not important when compared with control group and the materials can be used without effected on its property.
2- Statistically highly significant differences in mean impact strength test was observed of different groups when compared the untreated group (without any addition) with groups mixed with nystatin & fluconozal materials (the impact strength is affected (lowered) by mixing nystatin & fluconozale).

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