Original Study

SYNTHESIS OF 3-(2, 5-DIMETHYLFURAN-3-YL)-1H-PYRAZOLE-5(4H)-ONE, A NEW ANTIBACTERIAL COMPOUND AND USE IT FOR CREATING EXCELLENT ANTI-BACTERIAL PROPERTIES IN FLOWABLE COMPOSITES

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ABSTRACT

Background & Objective: - The objective of this study was to Synthesis of 3-(2, 5-Dimethylfuran-3-yl)-1H-pyrazole-5(4H)-one and Check its anti-bacterial properties in flowable composites

Method: - The Antibacterial activity of flowable resin composites containing 0–5 wt%. 3-(2, 5-Dimethylfuran-3-yl)-1H-pyrazole-5(4H)-one were investigated by using agar diffusion and direct contact tests on the cured resins.

Result: - According to our findings although the agar diffusion test reveals no significant difference between the groups, the direct contact test demonstrates that by increasing the 3-(2, 5-Dimethylfuran-3-yl)-1H-pyrazole-5(4H)-one content, the bacterial growth is significantly diminished ($p < 0.005$).

Conclusion: - Incorporation of 3-(2,5-Dimethylfuran-3-yl)-IH-pyrazole-5(4H)-one into flowable resin composites can be useful to prevent Streptococcus mutans activity.

Key Words: - Antibacterials Activity, Flowable Composites, Streptococcus Mutans.

Introduction

Usage of dental composites have been widely improved recently due to their advantages such as natural tooth color and easy handling over conventional material.[1] According to previous in vitro[2-5] and in vivo[2,6] studies, aggregation of bacteria and dental plaque were more on the surface of dental composites comparing to other dental materials so because of lack of antibacterial activity, these aggregations will cause more secondary caries on tooth. This phenomenon decreases the life of tooth restoration and eventually makes the restoration of replacement necessary.[7,8] Therefore more recent studies are focused on preparation of dental composites with antibacterial characteristics to prevent this kind of secondary caries. One of the methods for acquiring this purpose is use of antibacterial material in polymeric matrix. Antibacterial component will slowly release from the matrix and prevent the bacterial growth. An example of this kind of material is chlorhexidine and fluoride.[9-12] Despite of having strong antibacterial activity, they lack the proper prolonged drug release.[13] Another method is to use metal oxides for increasing the antibacterial activity of dental materials.[14-16] But using of these oxides in dental materials is limited because most of them will change the color of tooth.[17] Streptococcus mutans is one of the major causes of tooth decay.[18-21] Pyrazoles are a group of organic compounds that have been studied widely because of their biological activities.[22] They are the most important member of heterocyclic compounds that are used in pharmacological industries.[22-25] Pyrazoles have some useful characteristics such as anti-arthritic, Uricosuric, anti-inflammatory and antibacterial properties.[26] In this study, we synthesized pyrazole compounds, 3-(2,5-Dimethylfuran-3-yl)-IH-pyrazole-5(4H)-one. This compound has antibacterial properties and also its color is similar to the tooth color[27] that preserves tooth good appearance. We use this compound to provide antibacterial properties in dental material.

Method And Materials

Materials

Lithum bis (trimethylsilyl) amide (LiHMDS) Toluene, 3-acetyl-2,5-Dimethyl furan, Ethyl chloroformate, ethanol, acetic acid, ethyl chloroformate, ethanol, acetic acid, ethyl acetate, hydrazine hydrate and sodium sulfate were used manufactured by Sigma-Alderich. Dental restorative resin composite heliomolar flow prepared by Ivoclar vivodent. AG, FL-9494 schaan / Liechtenstein. Smutans PTCC 1683 (Persian Type culture-collection) received from IROST IRAN Co.

Methods

Synthesis of 3-(2, 5-Dimethylfuran-3-yl)-IH-pyrazole-5(4H)-one.

11 mmol of 1.0 M solution of LiHMDS in toluene was added to a solution of 3-acetyl-2,5-Dimethyl furan (10 mmol in toluene (15 mL)) using a syringe at 0°C under stirring. It stired in this temperature for 10 min and then 11 mmol of ethyl chloroformate was added quickly. Resulted reaction mixture was brought to room temperature during 10 min and was stired for another 10 min, and then 2 mL of acetic acid, 15 mL of ethanol and hydrazine hydrate (30 mmol) were added and refluxed for 15 min. mixture was concentrated to dryness under reduced pressure and dissolved in ethyl acetate. Organic impurities were washed by saturated NaCl solution dried over Na_2SO_4 and evaporated under reduced pressure. Final product was

purified by recrystallisation using ethanol. This process is shown in figure 1.

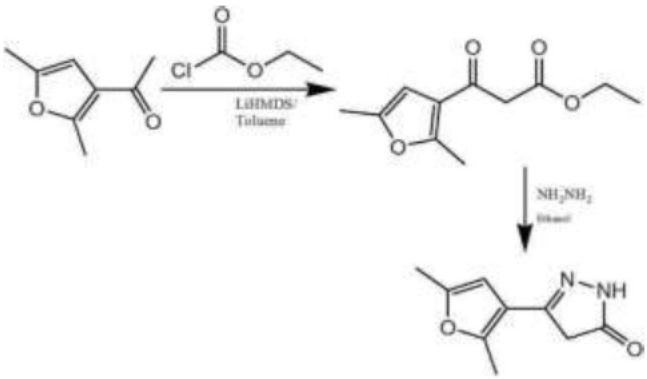

Figure 1: - Synthesis of 3-(2, 5-Dimethylfuran-3-yl)-1H-pyrazole-5(4H)-one

Conditions of Bacterial Growth

Standard strains of streptoccus mutans was used as the reference microorganism. It was grown overnight in Brain Heart Infusion medium (BHI).

Preparation of specimens:

Six groups of specimen were prepared by mixing of 3-(2,5-Dimethylfuran-3-yl)-IH-pyrazole-5(4H)-one with resin composite(Tetric flow, Ivoclar vivadent, USA) in 1,2,3,4 and 5 wt% and 0 wt% as the control group. Mixing was performed in a dark room for 15 min and in room condition using a spatula

Agar diffusion test

This test was used to investigate the effect of antibacterial activity in the composites containing 3-(2,5-Dimethylfuran-3-yl)-IH-pyrazole-5(4H)-one on bacterial growth. Discs with 2 mm thickness and 8 mm diameter were prepared using each of composites specimen groups. These discs were polymerized using a light cure device from two sides (bottom and top) for 40 s. In six groups, 200 µL of bacterial suspension was spread on blood agar and Then discs were placed on the surface of each of plates. After 24 hour of maintaining of plates in 37^0 C, inhibition zone diameter around each disc was measured. These tests were repeated 3 times each to ensure their accuracy.[28]

Direct contact test (DCT test)

This test performed to investigate the antibacterial properties of free surface of resins containing 3-(2,5-Dimethylfuran-3-yl)-IH-pyrazole-5(4H)-one. For this purpose walls of 500 µl microplates were covered by 200 µl of un-polymerized resin. Then resin layers were polymerized by a light cure device for 40s. 10 µl of 0.5 then McFarland standard solutions of Streptococcus mutans (about 10^6 bacteria) were added to each microplate and samples were kept in 37^0 C for 1hour. During this time (1 hour), bacteria were in direct contact with resin surface and the solvent was evaporated. Then 300 µl of BHI (Brain-heart infusion medium) were added to each microplate. Caps were completely closed and samples stored in 37^0 C. in periods of 24, 48, 72, 96 & 120 hours, 50 µl of the mixture (bacteria + BHI broth) was placed on culture medium and after 24 hours number of appeared bacteria colonies counted by using colony counter apparatus. This test were repeated 3 times for each test group to ensure their accuracy. The results were expressed as $\log_{10}$ (CFU). [Figure 2]

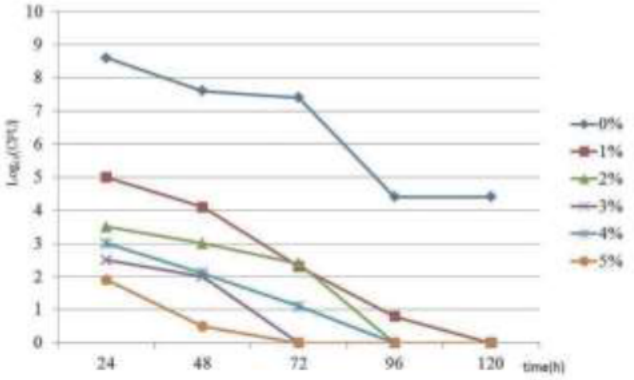

Figure 2: - Colony forming unit following direct contact between S.mutans and resin composites containing 0 – 5 wt. % 3-(2,5-Dimethylfuran-3-yI)-IH-pyrazole-5(4H)-one.

Data analysis

The data were analyzed by one-way ANOVA, and the tukey post hoc HSD multiple comparison test. The level of significance was determined as p=0.001.

Results

Agar diffusion test

There was no inhibition zone around the samples in agar medium containing s.mutans strain.

Direct contact test (DCT)

The results of bacterial colony count (colony forming unit,cfu) is shown in figure 2. It is clear that increasing of w% of 3-(2,5-Dimethylfuran-3-yl)-IH-pyrazole-5(4H)-one, antibacterial activity of composite increases significantly ($p<0.001$) and also time has a meaningful effect on antibacterial properties of resin.

Discussion

The agar diffusion test and the minimum inhibitory concentration are important traditional tests for evaluating and investigating of antibacterial properties and behavior of many pharmaceutical materials. Agar diffusion test works based on solvability of materials as tested material diffuses from the bulk surface and eliminates microorganisms. So this method cannot be used for the materials with low solvability in water. Giving that one of the most important and necessary properties of proper dental material is low water solvability, so the Agar diffusion test cannot be used here and it is not suitable test

method for dental material. I another words, DCT, has very low sensitivity toward test subject with solvability properties. Knowing that it is an efficient method to evaluate antibacterial properties, we can use it for investigating of antibacterial properties in materials with very low water solvability. As it is shown in Fig 2, DCT the results indicated that 3-(2,5-Dimethylfuran-3-yl)-IH-pyrazole-5(4H)-one compound gives excellent antibacterial properties to the resin that increases with time and in higher concentration. The antibacterial property of 3-(2,5-Dimethylfuran-3-yl)-IH-pyrazole-5(4H)-one reaches its maximum at 5% w and after 24 hour. Its non-toxic properties and also very its low aqueous solvability make 3-(2,5-Dimethylfuran-3-yl)-IH-pyrazole-5(4H)-one a very suitable material to be used in oral environment. In addition to mentioned advantages, 3-(2,5-Dimethylfuran-3-yl)-IH-pyrazole-5(4H)-one is white and it has the same color as tooth enamel. So it can improve the antibacterial properties of the dental composites and the same time preserving beauty characteristics of them.

According to previous researches, 3-(2,5-Dimethylfuran-3-yl)-IH-pyrazole-5(4H)-one probably uses the following mechanism to prevent bacterial growth. This compound is a derivative form of pyrazoles, one of most important nitrogenated heterocycle compound group. pyrazols prevent bacterial activity by possessing electron rich property and having non-covalent interaction with microorganism.

Next step in this research is to investigate the effect of this compound on mechanical properties of dental composite. We will conduct this research in future.

Conclusion

Within the limitation of this study it can be conclude that incorporation of 3-(2,5-Dimethylfuran-3-yl)-IH-pyrazole-5(4H)-one into flowable resin composites can reduce activity of Streptococcus mutans.

Acknowledgments

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