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Comparison Of Antibacterial Efficacy Of L-Arginine By Direct Contact Test- An In-Vitro Study

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Abstract: Dental caries, the most widespread oral disease globally, has shown a reduction in prevalence due to the introduction of fluoride. However, despite this decline, the overall prevalence of dental caries remains high. Recently, arginine has been introduced as an agent to combat tooth decay by targeting the reduction of Streptococcus mutans and acidity. The objective of this study was to evaluate the antibacterial effects of L-arginine against S. mutans through a direct contact test using two toothpastes: Colgate Total Whole Mouth Health (Group A) and Colgate Strong Teeth (Group B). Test samples were prepared by diluting 2mg of each toothpaste in 100 ml of distilled water. These solutions were added to a microtiter plate, and a 10-fold dilution was performed. BHI broth and a bacterial suspension of S. mutans were then added. Optical density values were measured hourly for 24 hours. ANOVA and Post hoc Tukey's test was applied for statistical analysis. After 24 hours, there was a significant difference within both groups, indicating that L-arginine exhibited antibacterial activity. There were no significant differences between the two groups at most concentrations, except at 1% where Colgate Strong Teeth toothpaste performed better. Within the limitations of the study it could be said that l-arginine has antibacterial activity but results have to be interpreted cautiously due to the in-vitro nature of the study.

Keywords: Anti-bacterial activity, Arginine, Direct Contact test

1. Introduction

A paste or gel substance that is used along with a toothbrush to preserve and improve oral health and esthetics is a toothpaste. Since their original invention thousand of years earlier, the formulation of toothpaste has undergone substantial modifications, evolving from straightforward simple mixtures of crushed eggshells or ashes to more complex formulations that include over 20 different ingredients. The usage of tooth powders rose during the 18th century, and by the 19th century, addition of glycerin to this powder to make a paste-like formulation was done. In 1914, the invention of fluoride led to an important breakthrough in toothpastes , which has been recognised as a major turning point in toothpaste history. [1]

Dentifrices are multipurpose items that employ several substances to achieve a variety of dental hygiene goals. By using abrasives and surfactants, they serve as agents that eliminate plaque and stains. They are made appealing by adding flavours and colours that people will want to use. Pyrophosphates are used to provide tartar control characteristics. Dentifrices also include fluoride and other ingredients with anti-inflammatory and desensitising qualities.[2]

Toothpastes , one of the most complex healthcare products, contains an aqueous humectant phase which comprises one or more abrasives which are suspended by hydrocolloid. This matrix consists of surfactant, various active therapeutic ingredients, flavoring agents, sweetening and coloring agents , preservatives, etc. Watson, C. A. "Synthetic hydrocolloids and dentifrices." *Journal of the Society of Cosmetic Chemists* (1970): 459-470. Their active ingredients include anti-plaque, anti-cariogenic, whitening, anti-odor agents as well as agents relieve dentinal hypersensitivity, and also remineralizing agents.

Toothpastes containing sodium fluoride were substituted by sodium monofluorophosphate, stannous fluoride, and amine fluoride[3]. Fluoride affects dental caries by a number of methods, including reducing bacterial

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metabolism, promoting remineralization, and preventing demineralization. Fluoride from drinking water may substitute hydroxyl or bicarbonate ions in the teeth's enamel to generate fluorapatite, a non-soluble, acid-resistant material[4]. Fluoride from external sources is transformed into hydrogen fluoride by bacterial acid, which prevents the formation of enzymes by its diffusion into cells. in the aqueous phase at low concentration , It can be adsorbed on the surface of enamel or dentin crystals thus , blocking acid's ability to dissolve the minerals. Solution-based topically applied sources promote remineralization by hastening the creation of a new surface on the partly demineralized subsurface crystals in the caries lesion. The new crystal surface veneer, which resembles fluorapatite, is not as soluble as the original carbonated hydroxyapatite tooth mineral.Fluoride is therefore a well-known anti-caries agent [5].

Fluoride has limitations in pathogenic conditions since its primary method of action does not particularly target dental plaque. One of the most recent developments in more successfully avoiding caries is the introduction of arginine. In toothpaste and other dental care products, arginine is added. After first being marketed to relieve the sensitivity of exposed tooth necks, arginine is now promoted as a caries-preventive agent. A peptide or protein is what makes up the arginine that the body takes in through food, saliva, and other host secretions.

The oral microbiota produces a wide range of proteases that can release the arginine into a form that many ADS-positive bacteria can internalise and metabolise. The ADS route generates ammonia, which lowers the pH of the cytoplasm and the surrounding environment and prevents the growth of cariogenic microorganisms[6–8]. The direct-contact test (DCT) was invented by Weiss. This test examines how directly physical contact between the test microorganisms and the subject material affects the kinetics of bacterial outgrowth. The agar diffusion method has been applied in many studies to assess the antibacterial capabilities of different toothpastes,[9–11] cements,[12,13]bonding agents,[14,15] and other goods, despite its limitations. Despite being used as the standard assay in the majority of these studies, the agar diffusion test (ADT) has some known flaws.

The semiquantitative nature of the ADT, the incapability to measure the activity of soluble components, the inability to distinguish between bacteriostatic and bactericidal effects, and the difficulties in controlling a large number of variables are some of the difficulties, according to Tobias[16].

The ADT requires careful standardization of inoculum density, medium content, agar viscosity, storage conditions of agar plates, size and number of specimens per plate, location and arrangement of specimens on plate, adequate contact between specimens and adjacent agar, and incubation time and temperature. Other methods, including growth curves[17], dilution in broth[18], modified cavity methods [19], and survival time [20,21], are all intended to get around some of the ADT's drawbacks. However, the fundamental premise of each of these assays, including the ADT, is to gauge the activity of those substances that are soluble and capable of diffusing into the surrounding media.

Regardless of the solubility and diffusibility of the antimicrobial components, the direct contact test (DCT) is utilized in this study to determine the impact of direct and intimate contact between the test microorganism and the tested material on microbial viability. Now the current growing trends motivated us to pursue this project. Thus the aim of this study was used to evaluate the antibacterial efficacy of two toothpastes that included L-arginine and fluoride using direct contact test.

2. Materials And Methods

Weiss et al first introduced DCT which is based on the turbidometric determination of bacterial growth in 96-well microtiter plates (Nunc, Roskild, Denmark). Using a temperature-controlled spectrophotometer, the kinetics of the outgrowth in each well was monitored continuously at 650 nm at 37°C every hour. (THERMOmax; Molecular Devices Corp, Sunnyvale, Calif). Auto-mixing was done before every reading, this ensured a homogeneous suspension of the bacterial cell.

In this study two toothpastes namely, Colgate Total Advanced Health toothpaste (Group A) (Colgate - Palmolive, India) and Colgate Strong teeth toothpaste (Group B) (Colgate - Palmolive, India) were assessed for their antibacterial activity. 2 mg of toothpaste was diluted in 100 ml of broth for each group. These test solutions were added to a 96 well microtiter plate and 10-fold dilution was done. Each sample was assessed in triplicate. 80-µL BHI broth along with 20-µL s.mutans culture was added to each well. Uncoated wells in the same microtiter plate served as the positive control while wells containing only the test material served as negative control. Outgrowth kinetics was recorded by measuring OD values using Spectrophotometer at 37°C. The plate was incubated at 37°C. OD values were recorded every hour till 24 hours and the graph was plotted.

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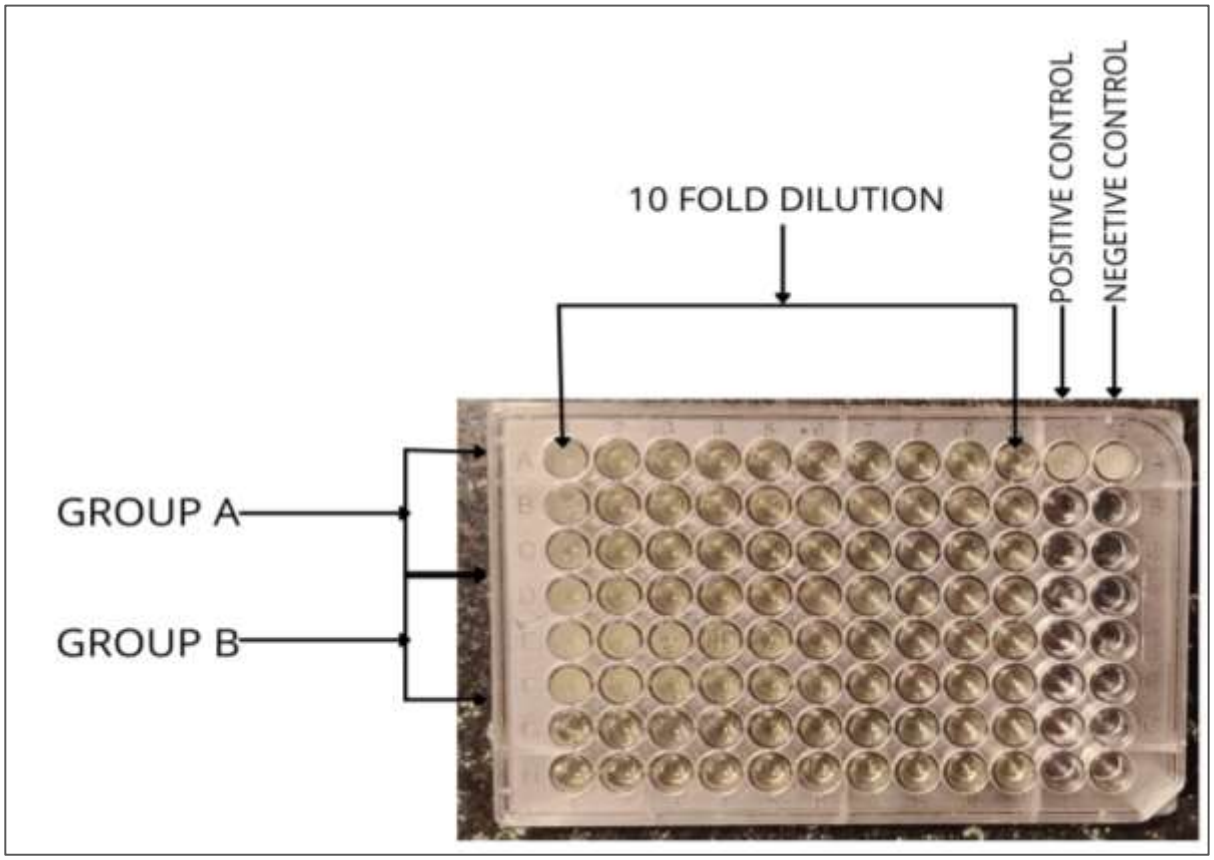

Fig 1. 96 well microtiter plate showing group A, group B, positive and negative control before Elisa reading.

3. Result

In the study direct contact test was done with two toothpastes over a period of 24 hours. Each point on the growth curve is the average of optical density measurements in 3 wells at any given time (0–24 hrs). ANOVA and Post Hoc Tukey's test were applied and SPSS software v.21 was used. Both the toothpastes were assessed at a concentration of 1%, 0.5%,0.25%, 0.125%,0.062%, 0.031%, 0.010%, 0.005%, 0.002% and 0.001%. In Group A for all 10 concentrations from baseline to 24 hours there was a significant difference whereas in Group B, except for 1% and 0.5% concentration, there was a significant difference from baseline to 24 hrs ($p > 0.05$). At 1% concentration, Group B showed significantly better antibacterial activity ($p < 0.05$) (Figure 2). On comparing both the groups at other concentrations there was no significant difference.

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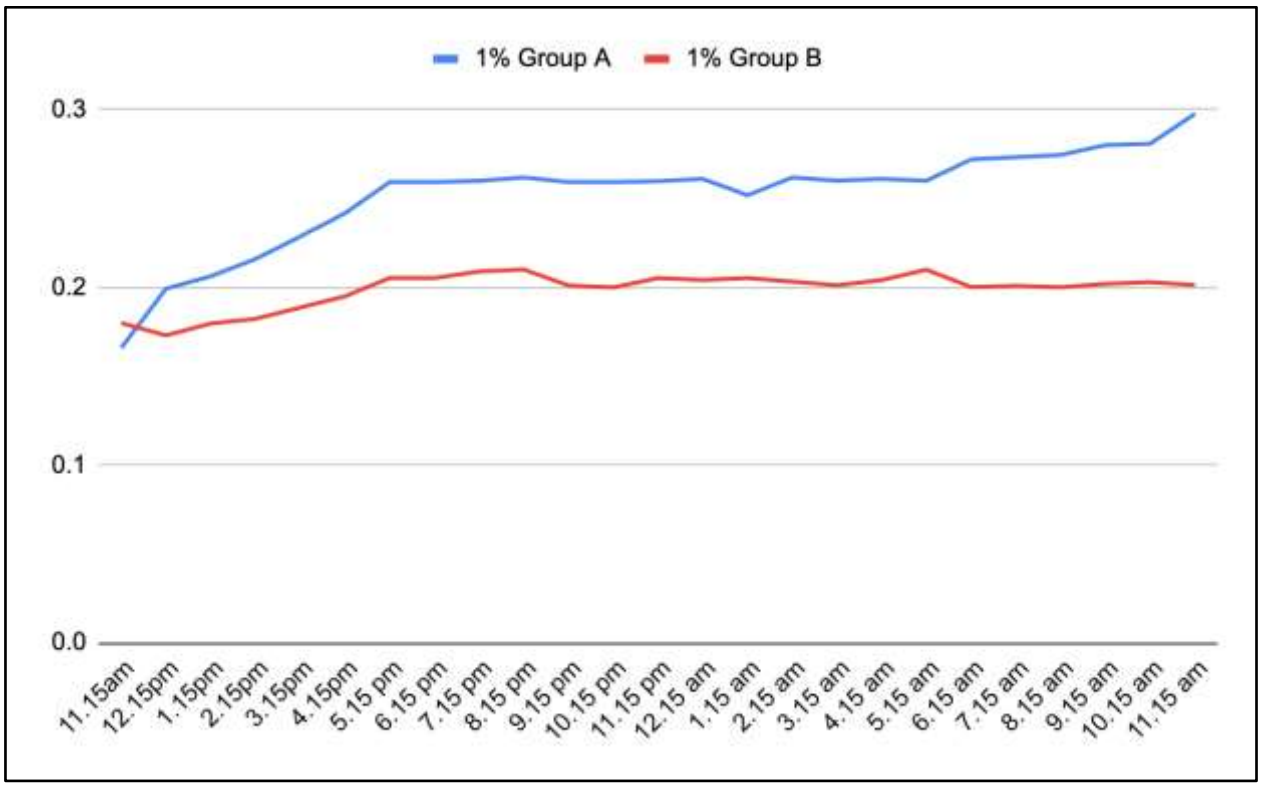

Fig 2. Growth Curve with mean optical density values every hour for Group A (Colgate Total Advanced Health toothpaste) and Group B (Colgate Strong teeth toothpaste)

4. Discussion

The global prevalence of dental caries is very well known. Newer materials are introduced in the market regularly to counter dental caries. This includes toothpaste containing various anticaries agents, mouth rinses and gums. The role of microbes and their by-products in initiation and progression of caries is very well established[22]. Streptococcus mutans has been identified as a primary microorganism for the initiation development of dental caries in humans[23]. Hence in this study we wanted to assess the antibacterial activity of L-arginine in dentifrices against s.mutans.

Recently, arginine has been introduced as an additive in toothpaste and other dental care products that contain fluoride. Initially, it was marketed to address tooth sensitivity in exposed tooth necks. However, it is now being promoted as a preventive agent against tooth decay. Arginine is an amino acid naturally found in various food products and saliva. When metabolized by arginolytic bacteria, it generates substances similar to ammonia, leading to an elevation in the pH level within the oral biofilm. As a result, this counters the acidic conditions that facilitate the growth of acid-resistant bacteria[24].

Colgate Strong Teeth Toothpaste contains Calcium Carbonate, Arginine, Silica, Sorbitol, Titanium Dioxide, Potassium Nitrate, Sodium Monofluorophosphate, Sodium Silicate, SLS and Sodium Saccharin. Sorbitol, Silica, Sodium Lauryl Sulfate, Pvm/Ma Copolymer (Gantrez), Carrageenan, Gum, Arginine ,Sodium Hydroxide, Titanium Dioxide, Sodium Saccharin, Triclosan and Sodium Fluoride are present in Colgate Total Advanced Health toothpaste.[25] Both these toothpastes contain equal amount of l-arginine.

In the present study it was found that at 1% concentration Colgate strong teeth toothpaste had better antibacterial action compared to Colgate Advanced Health toothpaste while at all other concentrations both groups had similar antibacterial action. Within both the groups there was a significant antibacterial activity after 24 hours. The results of this study are in accordance with an in-vitro study by X. Zheng et al, 2015 where combinatory effect of arginine and fluoride on s.mutans was found to be significant[26].

Dental biofilm refers to the colonization of bacteria on tooth surface, artificial tissues or implants, where they become embedded within the extracellular matrix consisting of polymers such as polysaccharides, proteins, and DNA[27]. Among the microorganisms present in plaque, Streptococcus mutans accounts for approximately 30%

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and is a primary contributor to dental caries in humans[28]. This is attributed to the bacteria's capability to form biofilms, metabolize various carbohydrates into organic acids, and thrive and carry out acidic growth and metabolism[29].

It is important to acknowledge that the oral microbiota is a constantly changing group of numerous microbial species that colonize various oral regions in a unique manner [30]. The findings derived from the specific microbial community analyzed in this study only demonstrate a tendency of arginine to influence microbial composition.

5. Conclusion

Within the limitations of the study, it can be concluded that although Colgate strong teeth toothpaste showed better antibacterial activity at 1% , both the dentifrices showed excellent antibacterial activity over the period of 24 hours.

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