Antimicrobial Effect of Alpha Lipoic Acid Against Oral Microorganisms.

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Abstract: Aim: This study was aimed to assess the antibacterial effect of alpha lipoic acids against various oral microorganisms.

Background: Oral microorganisms are responsible for oral related diseases. Oral microorganisms implicated in the etiology of dental caries, stomatitis, gingivitis and periodontitis. Gradual increase in the rate of resistance to antibiotics leads to increase in oral pathologies.

Materials and method: 100 mg of alpha lipoic acid was measured and dissolved in 2 ml of ethanol. 250 ml of Muller Hinton Agar (MHA) was prepared, sterilised, poured onto the petri plates. After solidification each MHA plates were swabbed by overnight culture of enteric organisms such as Staphylococcus aureus, Streptococcus mutans, Pseudomonas species, Enterococcus faecalis, Escherichia coli, Candida albicans. Using a well cutter 3 wells were formed on each MHA plate. In each well, alpha lipoic acid was added at the concentration of 30, 60 and 90 ul and the petri plates were kept for incubation at 37 °C for 24 h. The zone of inhibition was recorded in each plate.

Result: Alpha lipoic acid prominently inhibited the growth of all the organisms invested. It possesses inhibitory antimicrobial effects even at low concentrations and it has shown maximum inhibitory antimicrobial activity at higher concentrations. Candida albicans was found as highly susceptible to alpha lipoic acid.

Conclusion: The potential use of alpha lipoic acids provided in a local delivery vehicle to infected sites in the oral cavity, could be considered as an additional therapeutic approach to improve oral health.

Key words: Alpha lipoic acid, oral microorganisms, oral diseases, oral health, Candida albicans

INTRODUCTION

The oral cavity contains over and above 600 bacterial species.(Karthiga, Rajeshkumar and Annadurai, 2018) Oral microorganisms are present generally in the form of a biofilm and sustain ecological equilibrium with the host body. Oral microbiota has also been considered as a potential biomarker of human diseases.(Radhika, Ezharasan and Gopinath, 2017) The ecological imbalance of biofilm manifested as various oral related infectious diseases that include tooth decay, dental caries, gingivitis, oral thrush, periodontitis, periconoritis, periodontal diseases and craniofacial bone osteomyelitis. Oral microorganisms are implicated with many systemic diseases including cancer, diabetes mellitus, rheumatoid arthritis, cardiovascular diseases and preterm birth.(Gowtham et al., 2018) Dental plaque is an important etiologic factor in dental caries. Several strains of oral streptococci, staphylococci and lactobacilli are capable of formation of dental plaque which play an important role in development of dental plaque, dental caries and periodontal disease.(Rajeshkumar, Venkat Kumar, et al., 2018) Recently there has been an increased resistance of these microbial organisms against present antimicrobial agents and adverse effects of these drugs is a major concern.(A et al., 2017) Therefore there is a need to develop alternative antimicrobial drugs for the treatment of infections to counteract the resistance and to minimize the adverse effects.(Rajeshkumar, Agarwal, et al., 2018) Several components have been investigated as promising agents to prevent oral diseases especially dental plaque and dental caries. (Rj et al., 2017)

Lipoic acid, also known as α-lipoic acid and alpha lipoic acid (ALA) and thioc acid is an organosulfur compound derived from caprylic acid (octanoic acid).(Rozo, 1974) Alpha lipoic acid (ALA) is an eight-carbon disulfide compound and functions as a natural cofactor in pyruvate and α-keto dehydrogenase complexes.(Dong et al., 2020) ALA is made in animals normally, and is essential for aerobic metabolism.(Menon et al., 2018; Gheena and Ezharasan, 2019) It is also manufactured and is available as a dietary supplement in some
countries where it is marketed as an antioxidant, and has good antimicrobial activity and is available as a pharmaceutical drug in other countries. (Fuchs, 1997; Nandakumar et al., 2016). Alpha lipoic acid and its reduced form, dihydrolipoic acid, act as potent antioxidants that scavenge free radicals. (Ezhilarasan, Sokal and Najimi, 2018) The antioxidant role of Alpha lipoic acid has been implicated in hepatitis, diabetes, atherosclerosis, urolithiasis, HIV infection and also in the treatment of acute liver poisoning, liver cirrhosis, heavy metal poisoning, and other liver pathologies. (Rozo, 1974)) (Berkson, 2010) Oral administration of ALA was investigated against chloroquine-induced toxicity in Wistar rats by Pari and Murugavel for its possible hepatoprotective effect. (Institute and National Cancer Institute, 2020) Moreover, Alpha lipoic acid exhibited a greater antimicrobial effect when compared with silymarin, a plant-derived drug. (Inserm and INSERM, 2020) Alpha Lipoic Acid is a promising alternative antimicrobial agent that is increasingly being used in clinically applied biomaterials to inhibit microbial colonisation and subsequent infection. (Leysen and Aerts, 2016)(Ezhilarasan, 2018) They are already clinically used in wound dressings, catheters and implants, for prophylactic or therapeutic treatment. (Ajith, 2020) It has also been reported that Alpha Lipoic Acid can result in the induction of oxidative damage and inflammatory lesions in human gingival fibroblast cells. (Institute and National Cancer Institute, 2020)(Ezhilarasan, Lakshmi, Nagaich, et al., 2017) As with any new therapeutic, it is essential to investigate not only the targeted efficacy but the safety of lipoic acid on contacted cells in a clinical scenario. Primary human cells are the gold standard for in vitro testing of new therapeutics and in this study primary human derived gingival fibroblasts were used for examining the potential of lipoic acid for intra-oral topical application and as a representative in vitro system. (Nakano and Sano, 1955) (Ezhilarasan, Lakshmi, Vijayaragavan, et al., 2017; Mehta et al., 2019) With this in mind, the present study investigated the antimicrobial effects of alpha lipoic acid on a range of oral microorganisms. Our team has rich experience in research and we have collaborated with numerous authors over various topics in the past decade (Deogade, Gupta and Agrawal, 2018; Ezhilarasan, 2018; Ezhilarasan, Sokal and Najimi, 2018; Jeewanandam and Govindaraju, 2018; J et al., 2018; Menon et al., 2018; Prabakar et al., 2018; Rajeshkumar, Kumar, et al., 2018; Vishnu Prasad et al., 2018; Wahab et al., 2018; Dua et al., 2019; Duraisamy et al., 2019; Ezhilarasan, Apoorva and Ashok Vardhan, 2019; Gheena and Ezhilarasan, 2019; Malli Sureshbabu et al., 2019; Mehta et al., 2019; Panchal, Jeewanandam and Subramanian, 2019; Rajendran et al., 2019; Rajeshkumar et al., 2019; Ramakrishnan, Dhanaalakshmi and Subramanian, 2019; Sharma et al., 2019; Varghese, Ramesh and Veeraiyan, 2019; Gomathi et al., 2020; Samuel, Acharya and Rao, 2020)

MATERIALS AND METHODS
Enterococcus faecalis, Streptococcus mutans, Staphylococcus aureus, Pseudomonas species, Escherichia coli and other oral pathogens was isolated from patients of Saveetha Dental College and Hospitals, Chennai, India. Organisms were cultured using standard methodology. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) was studied using a well diffusion method. The agar well diffusion method was used to determine the antibacterial activity of ALA.

Clinical isolates
Enterococcus faecalis, Streptococcus mutans, Staphylococcus aureus, Candida albicans, pseudomonas species.

Preparation of sample
100mg of alpha lipoic acid was measured and dissolved in 2ml of ethanol.

Determination of Minimal Inhibitory Concentration/ minimum bactericidal concentration (MIC/MBC)
250ml of Muller Hinton Agar was prepared, sterilised, poured on to the petri plates. After solidification each MHA plates were swabbed by overnight culture of enteric organisms such as Staphylococcus aureus, Streptococcus mutans, Pseudomonas species, Enterococcus faecalis, Escherichia coli, Candida albicans. Using a well cutter 3 wells were formed on each MHA plate. Different concentrations of lipoic acid were tested against Staphylococcus aureus, Streptococcus mutans, Escherichia coli, Enterococcus species and Pseudomonas species. The fresh bacterial suspension was dispersed on the surface of Muller Hinton agar plates. In each well, alpha lipoic acid was added in the concentration of (30, 60 and 90 ul) and the petri plates were kept for incubation at 37 °C for 24 hours. The antibiotics were used as a positive control. Zone of inhibition was recorded in each plate.

RESULT AND DISCUSSION
We evaluated the MIC/MBC of ALA against different clinical isolates. In this study, lipoic acid shows inhibitory efficacy on clinical isolates such as Enterococcus faecalis, Streptococcus mutans, Pseudomonas species, Staphylococcus aureus, Candida albicans against Among the isolates tested, ALA has the potential antimicrobial action against Candida albicans ALA also inhibited the bacterial growth of Enterococcus faecalis. Streptococcus mutans, Pseudomonas species, Staphylococcus aureus. Dental caries is one of the most
Malnutrition and destruction of the hard tissues of teeth. (Anitha and Ashwini, 2017) It is a well-known fact that orodental pathogens play a pivotal role in the development of caries. The dental profession has, of course, been aware of this for many years and consequently has developed preventive and therapeutic regimens for these diseases based on mechanical removal of the biofilms. (Ashwini, Ezharasran and Anitha, 2017) Candidiasis is a fungal infection due to any type of Candida when it affects the mouth, it is commonly called thrush. Signs and symptoms include white patches on the tongue or other areas of the mouth and throat. Here our lipoic acid showed maximum inhibitory effect towards Candida albicans. This report shows that plants remain an important source for the development of new chemotherapeutic agents. The first step towards this goal is the in vitro antibacterial activity. Several plants have been investigated previously for their antimicrobial efficacy and came with promising results.

Oral microorganisms are known for their pathogenesis in tooth decay, gingivitis, periodontitis and their ability to cause tooth loss. Periodontal diseases and dental caries are two main common dental pathologies affecting humankind. (Nandakumar et al., 2016) These conditions are caused by plaque forming bacteria and yeast, which reside in the oral cavity. Periodontal diseases have mainly been associated with Actinomyces, Actinobacillus, Streptococcus and Candida species. Candida albicans is not cariogenic, but was included in this study because it is a pathogenic microorganism causing oral thrush particularly in immunocompromised individuals. (More et al., 2008)

It has been reported that systemic Candida albicans infections are fatal in 42% of cases, despite the use of antifungal therapies and Candida albicans is the fourth most common infection in hospitals. Fluconazole is a potent and broad spectrum antifungal agent. It is active against many candida species. (Lakshmi et al., 2015; Sharma et al., 2019) However it has few side effects and this drug has developed resistance to Candida albicans over time. In the present study, Alpha lipoic acid inhibits the growth of Candida albicans in its low concentration indicating the fact that this drug can be a therapeutic alternative for Candida albicans infection.

Chao shi, Yi sun et al., 2016 proved that LA has inhibitory effect against several C. sakazakii strains, most of which were isolated from infant food. (Shi et al., 2016) Joshi et al., 2014 showed Several reports have described the effect of natural antibacterial substances, including blueberry proanthocyanidins and commercial blueberry juice, (Joshi, Howell and D’Souza, 2014) Lee & Jin et al., 2008 carvacrol, thymol, caprylic acid, citric acid, and vanillin (Lee and Jin, 2008) Amalaradouj, Hoagland, Venkitanarayanan, et al., 2009 trans-cinnamaldehyde (Lee and Jin, 2008; Amalaradouj, Hoagland and Venkitanarayanan, 2009) Kim, Kim, & Jung, et al. 2008 water-soluble muscadine seed extracts and Kim, Weng, Silva, Jung, Marshall, et al., 2010 red muscadine juice. However, in many cases, the antimicrobial effects of these plant substances were measured with dehydrated infant milk formula (RIMF) mode. (Kim, Kim and Jung, 2008)

Ohta et al., 1995 demonstrated the antibacterial activity of α-lipoic acid against methicillin-resistant S. aureus. The possibility of the therapeutic use of lipoic acid as an antibacterial agent should be explored. (Lacey and Lord 1981) (Perumalsamy et al., 2018) α-lipoic acid is generally considered to have low toxicity, so it may potentially be administered to patients infected with MRSA as a dietary treatment (Ohta et al., 1995). Cooper et al. (1985) described the antibacterial activity of lipoic acid against B. subtilis and Vibrio parahaemolyticus. The marine bacterium, V. parahaemolyticus, is a major cause of gastroenteritis in countries where large amounts of sea fish and its products are consumed (Cooper et al. 1983). Lipoic acid had inhibitory activity against spores of Clostridium botulinum, Clostridium sporogenes and B. cereus. (Cooper et al., 1983)

Vaishali and Geetha et al., showed the antimicrobial effect of orange peel oil against orodental pathogens where they found that dandelion has an effect on Enterococcus faecalis and Streptococcus salivarius. (Vaishali and Geetha, 2018) Nithya Karphagam et al., determined the antibacterial activity of lemongrass oil using agar well diffusion technique against the following oral pathogens: Streptococcus mutans, Enterococcus faecalis and lactobacillus acidophilus. They found that lemongrass oil has antibacterial activity against these pathogens especially against Streptococcus mutans. (Karphagam et al., 2016) Piper nigrum has a broad antibacterial activity and it can be incorporated into medication for topical antifungal therapy. (Karthikeyan, Geetha and Thangavelu, 2019) The anti inflammatory activity of bay leaf to inhibit protein denaturation and its a cause of inflammation. The anti inflammatory effect of the bay leaf was comparable to reference analgesics and non steroid anti inflammatory drugs. (M, Geetha and Thangavelu, 2019) Our institution is passionate about high quality evidence based research and has excelled in various fields (Pc, Marimuthu and Devadoss, 2018; Ramesh et al., 2018; Vijayashree Priyadharsini, Smiline Girija and Paramasivam, 2018; Ezharasran, Apoorva and Ashok Vardhan, 2019; Ramadurai et al., 2019; Sridharan et al., 2019; Vijayashree Priyadharsini, 2019; Chandrasekar et al., 2020; Mathew et al., 2020; R et al., 2020; Samuel, 2021)

CONCLUSION
The lipoic acid showed potential antimicrobial effect against E. faecalis, S.aureus, S.mutans, C.albicans, and Pseudomonas species. Dental caries are reported to be predominantly caused by these microbes. The potential use of alpha lipoic acids provided in a local delivery vehicle to infected sites in the oral cavity, could
be considered as an additional therapeutic approach to improving oral health. Hence, it is suggested that the antibacterial efficacy of Alpha lipoic acid should be watched for its beneficial effects against dental caries. However, further studies can be done based on alpha lipoic acid to show further results.

*Streptococcus mutans*  
*Pseudomonas species*  
*Enterococcus species*  
*Staphylococcus aureus*  
*Candida albicans*

**Fig. 1:** Antimicrobial effect of alpha lipoic acid (ALA) against oral pathogens. The *Candida albicans* shows the maximum growth inhibition.
Fig.2: Antimicrobial effect of alpha lipoic acid (ALA) against oral pathogens. Zone of inhibition was measured in mm for ALA for the concentrations of 30, 60 and 90 ul.

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