

Comparative Evaluation of Antimicrobial Efficacy of Simvastatin Paste and Aloe Vera Paste as a Pulpotomy Medicament- An In-vitro Study

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ABSTRACT

Introduction: Pulpotomy is the complete removal of the coronal part of the dental pulp, followed by the application of an appropriate dressing or medicament to promote healing and maintain the viability of the teeth in the dental arch. Aloe vera has anti-inflammatory, antibacterial properties which can be used as a pulpotomy medicament. Simvastatin, a lipid-lowering medication has recently emerged as a potential medicament in regenerative dentistry. It has an anti-inflammatory and antioxidant properties. Hence, it can be used as a pulpotomy medicament.

Need of the study: The present study can provide evidence regarding the use of Aloe vera and Simvastatin in pulpotomy with respect to their bactericidal activity, biocompatibility and promoting root pulp healing.

Aim: To evaluate and compare the antimicrobial efficacy of Aloe Vera paste and Simvastatin paste as pulpotomy medicament against *Pseudomonas aeruginosa* and *E. faecalis*

Materials and Methods: An in-vitro study will be conducted in Department of Paediatric and Preventive Dentistry at Sharad Pawar Dental College, Wardha, Maharashtra, India, in which Aloe Vera paste and Simvastatin paste will be prepared in an aseptic environment. The antimicrobial activity of simvastatin paste and Aloe Vera paste against *E. faecalis* and *pseudomonas* will be assessed using the agar diffusion method. It will be cultured overnight in brain heart infusion broth. On blood agar media, *pseudomonas aeruginosa* will be inoculated. 4 mm wells will be punched out on plates with a puncher and will be instantly loaded with freshly customised test materials. The agar plates shall then be incubated in an incubator at 37°C for 24 hours and 72 hours before being analysed. Statistical analysis will be done using Chi-square test, ANOVA test and multiple comparison 2k test.

Keywords: Pulpotomy material, Regeneration, Statin

INTRODUCTION

In pulpotomy coronal portion of the dental pulp is completely removed and the appropriate dressing or medicament is applied to hasten healing and maintain the health of teeth on dental arch. Several pulpotomy materials, including calcium hydroxide, ferric sulphate glutaraldehyde, formocresol enhanced mixture cement, and mineral trioxide aggregate have been studied in radiographic and clinical trials to determine their mechanism of action, clinical symptoms, and benefits [1]. Properties of ideal pulpotomy material would be bactericidal and biocompatible, promote root pulp healing, and be compatible with root resorption physiological process. However, the appropriate pulpotomy medicament that meets all of the parameters has yet to be identified [2]. Many natural products promise to have a major impact and appear to be a feasible alternative for formocresol, including Curcuma longa, turmeric, Nigella sativa, Thymus vulgaris, Honey, Allium sativum oil, Aloe Vera, and Acemannan [3].

Aloe Vera is a succulent, elongated tropical and subtropical plant with a shrubby appearance. Aloe Vera Barbadensis Miller is a member of the Liliaceae family, which is frequently utilised for therapeutic purposes [4]. It has been used as a home cure for burn wounds for a long time. As a result, it's known as the "burn plant." Aloe Vera has a variety of dermatological uses, including inflammation treatment, wound healing, and antibacterial activity, particularly in the treatment of thermal burns, radiation burns, and frostbite. It promotes macrophage recruitment, collagen formation, and wound contraction, among other wound healing processes [4].

In Dentistry, Aloe Vera plays a major role in the treatment of oral lichen planus, oral submucous fibrosis, recurrent aphthous stomatitis, alveolar osteitis, periodontitis etc., [4]. In Paediatric Dentistry Kriplani R et al., performed an in-vitro experiment using Aloe Vera in powdered form and proved that Aloe Vera with sterile water has superior antimicrobial activity as a root canal filling material [4]. Aloe Vera has anti-inflammatory, antibacterial, immunomodulatory, antiviral, and pain-relieving activities in nature [5].

Simvastatin, a strong lipid-lowering medication (3hydroxy, 3methylglutaryl co-enzyme) A (HMG CoA) reductase inhibitor, has recently emerged as a potential medicament in regenerative dentistry [2]. Statin enhances bone formation by improving osteoblast function and suppressing osteoclast function via the Bone Morphogenic Protein (BMP)-2 pathway. As a result, statins may help odontoblasts function efficiently, resulting in better dentin production [6]. It has been proven to have regenerative impacts on dental pulp stem cell, odontoblastic differentiation and the generation of reparative dentin [7,8]. Statins play a vital role in oral cancer by inhibiting the growth, metastasis, invasion etc. It helps in wound healing, periodontal treatment and pulpotomy treatment. Simvastatin has anti-inflammatory, antioxidant properties, immunomodulatory action, antifungal, antiviral and antithrombotic properties [9]. Aripirala M et al., performed an in-vivo study on diode laser and Simvastatin gel as a pulpotomy material [2]. Whitaker EJ and Alshammari A, performed an in-vitro study on bacteriostatic effects of simvastatin solution against *Streptococcus* and prove that simvastatin inhibits the growth of the test organisms [10].

Aloe Vera and Simvastatin has antimicrobial properties. Simvastatin has not been studied in the paste form as a pulpotomy material

in literature. In past studies there is only one literature which has evaluated clinically the efficacy of pure simvastatin as a pulpotomy agent in gel form [2]. Instead of simvastatin in gel form simvastatin in paste form will show good results because gel form might get displaced from its place in the tooth [2]. So, the aim of this study is to evaluate and compare the antimicrobial efficacy of Aloe Vera paste and Simvastatin paste as a pulpotomy medicament against *Pseudomonas aeruginosa* and *E. faecalis*.

The objectives of the study are to evaluate the antimicrobial efficacy of Aloe Vera and Simvastatin paste as pulpotomy medicament against *Pseudomonas aeruginosa* and *E. faecalis*. Second, to compare the antimicrobial efficacy of Aloe Vera and Simvastatin paste as pulpotomy medicament against *Pseudomonas aeruginosa* and *E. faecalis*. Third is to evaluate the antimicrobial efficacy of Aloe vera and Simvastatin paste with calcium hydroxide against *Pseudomonas aeruginosa* and *E. faecalis*. And last objective is to compare the antimicrobial efficacy of Aloe vera and Simvastatin paste with calcium hydroxide against *Pseudomonas aeruginosa* and *E. faecalis*.

REVIEW OF LITERATURE

The main cause of pulpal and periradicular diseases are microorganisms and their by-products [11]. The major goal of pulpotomy treatment is to remove the infected coronal portion of the pulp and thereby decreasing the microbial load in the affected tooth [4]. The mucilaginous gel produced by the parenchymatous cells in the leaf pulp of Aloe Vera has been utilised for a variety of medicinal applications since ancient times. Aloe Vera materials have been suggested to have a direct antimicrobial effect. It has been demonstrated to have anti-inflammatory, regenerative, nutritional, and antibacterial effects, among other medicinal characteristics [12]. Gala-Garcia A et al., conducted one investigation on the effects of Aloe Vera on rat pulpal tissue [13]. Aloe Vera which is 4 years grown is taken in the study. Pulpal tissue was manually treated with Aloe Vera 1-7 days after therapy the Aloe Vera-treated group displayed a noticeable acute-moderate inflammatory infiltration. When compared to Ca(OH)₂ therapy, pulp tissue returned to normal after 14-30 days; both treatment groups triggered reparative dentin and the establishment of a full bridge [13].

Microbiological study which is conducted by Kriplani R et al., in which Aloe Vera was compared to several root canal materials like calcium hydroxide Zinc Oxide Eugenol (ZOE) calcium hydroxide with idoform etc., As a result, Aloe Vera had a high inhibitory effect on aerobic gram-positive microbes and a moderate inhibitory effect on aerobic gram negative microorganisms. ZOE+Aloe Vera had a moderate inhibitory effect on both groups of test microorganisms, whereas ZOE had a moderate inhibitory effect on both groups of test microorganisms. As a result, incorporating Aloe Vera into ZOE improves the antibacterial properties of the ZOE-Aloe Vera combination when compared to ZOE alone [4]. As mentioned in Kriplani R et al., Aloe Vera will be used in present study but, it will be in paste form.

In the present study, Aloe Vera harvested after 3 years of growth will be taken because of increase in effectiveness of the plant, while after 12 years of Aloe Vera growth it loses some of its nutritional potency [4]. Therefore, in the present study, Aloe Vera after 12 years of growth will be excluded from the study. Statins have distinct anti-inflammatory, angiogenesis-inducing, osteoblastic, and odontoblastic differentiation capabilities. Simvastatin improves osteoblast function and suppresses osteoclastic activity, resulting in bone growth when applied locally [14]. Stein D et al., investigated a scientific study on effects of simvastatin on growth and inflammation of mandibular bone. Female rats were given different doses of simvastatin (0.1, 0.5, 1.0, 1.5, or 2.2 mg) through methylcellulose gel in a polylactic acid membrane on the lateral side of the jaw. When 0.5 mg simvastatin was used instead of gel, bone area

increased by 45 percent, and clinical inflammation was minimised when compared to the high simvastatin dose. The 0.1 mg dose of simvastatin did not result in considerable bone development. As a result, Simvastatin was lowered from 2.2-0.5 mg, which decreased swelling to a clinically manageable level without affecting bone development potential [15]. Present study will use the similar simvastatin material which is mentioned in the study of Stein D et al., but it will convert into paste form [15].

A randomised clinical experiment was conducted by Aripirala M et al., to assess and compare the clinical and radiographic efficacy of the diode laser and simvastatin gel in pulpotomy of carious primary molars. The study was performed on 90 patients 45 with diode laser and 45 with simvastatin gel. Clinical and radiological follow-up evaluations were done at 3 and 12 months. In results, abscess was diagnosed in 9 molars of the diode laser group and 8 molars of the simvastatin gel group after 3 months. Two more molars in the diode laser group and 1 more in the simvastatin gel group had abscess at 12 months. Simvastatin gel shows more clinical success rate than diode laser pulpotomy. Author concluded that simvastatin gel can be recommended as a potential pulpotomy medicament in primary molars [2]. As efficacy of simvastatin gel is proven, present study will try to prove its efficacy in paste form.

MATERIALS AND METHODS

This study will be conducted in Department of Paediatric and Preventive Dentistry at Sharad Pawar Dental College, Wardha, Maharashtra, India. Ethical approval for the study was obtained from the Institutional Ethics Committee (IEC ref no. DMIMS(DU)/IEC/2022/755).

Inclusion criteria: The inclusion criteria consists of natural extract of Aloe Vera from the Liliaceae family harvested after 3 years of growth, from botanical garden, 97% pure simvastatin powder from sigma Aldrich company and *Pseudomonas aeruginosa* and *E. faecalis* strain.

Exclusion criteria: Aloe Vera plant which is older than 12 years will be excluded from the study.

Study Procedure

The study will consist of four groups. The first group will contain pure Aloe Vera paste, for which Aloe Vera leaves will be collected and freeze-dried. After drying the powder, it will be mixed with 1.5 mL saline water and converted into paste form. For the second group, Aloe Vera paste with 1 mL propylene glycol shall be mixed with 0.17 g of Calcium hydroxide [4]. For preparing simvastatin paste, pure simvastatin (97 percent pure) [10] will be procured in powder form mixed with distilled water and converted into paste form. Third group will consist of Simvastatin paste which will be prepared under an aseptic environment using a vortex unit to combine 0.03 mg simvastatin powder with 10 mL distilled water and a carrier paste; and for group four, 0.03 mg of calcium hydroxide shall be added to the above-mentioned simvastatin paste [2]. All the groups shall be tested against *Pseudomonas* and *E. faecalis*.

In the microbial assay, the antimicrobial activity of simvastatin paste and Aloe Vera paste against *E. faecalis* and *pseudomonas* will be assessed using the agar diffusion method. *E. faecalis* will be obtained as a bacterial stock culture, and will be cultured overnight in brain heart infusion broth. *Pseudomonas aeruginosa* will be inoculated on blood agar media. Wells of 4 mm will be punched out on 12 plates with a puncher under an aseptic environment, according to the manufacturer's specifications, and instantly loaded with freshly customised test materials. The Agar plates will then be incubated in an incubator at 37°C for 24 hours and 72 hours before being analysed. By using digital calliper, the diameter of microbial inhibition zones, of this pulp capping material around each well to the nearest size in mm will be measured at 24 hours and 72 hours to assess

their antimicrobial efficacy against *E.faecalis* and *pseudomonas* [16]. The obtained data will be tabulated and analysed statistically.

Primary outcome: Simvastatin paste and Aloe Vera paste will prove to be more efficient as pulpotomy medicament and will be able to provide Antimicrobial efficacy against *Pseudomonas aeruginosa* and *E.faecalis*.

STATISTICAL ANALYSIS

Statistical analysis will be done using software SPSS Version 27.0 and Graph pad Prism 7.0 version where, ($p < 0.05$) will be considered as level of significance. The findings will be analysed statistically using Chi-square test ANOVA test and multiple comparison 2k test.

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