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Disinfection potential of glycerol, chitosan and clove oil along with triple antibiotic paste against root canal microorganisms

[1]Dr. P Shanti Priya, Senior lecturer, Department of Conservative Dentistry and Endodontics, Panineeya Institute of Dental Sciences, Hyderabad, Telangana.

[2]Dr. Raji Viola Solomon, Professor, Department of Conservative Dentistry and Endodontics, Panineeya Institute of Dental Sciences, Hyderabad, Telangana.

[3]Dr. Ashish Jain, Reader, Department of Conservative Dentistry and Endodontics, Panineeya Institute of Dental Sciences, Hyderabad, Telangana.

[4]Dr. Divya Talla, Post graduate, Department of Prosthodontics, Panineeya Institute of Dental Sciences, Hyderabad, Telangana.

Corresponding Author: Dr. P Shanti Priya, Senior lecturer, Department of Conservative Dentistry and Endodontics, Panineeya Institute of Dental Sciences, Hyderabad, Telangana.

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Abstract

Aim: To determine the antimicrobial efficacy of triple antibiotic paste (TAP) with different vehicles like glycerol, chitosan and clove oil against root canal microorganisms such as Enterococcus faecalis and Candida albicans.

Materials & Methods: The antimicrobial effect of TAP which is an intracanal medicament was studied against E. faecalis and C. albicans with antibiotic sensitivity test using Agar well diffusion method over a period of 1,3, and 7 days. TAP was mixed with four different vehicles such as group I(Saline+ TAP), group II(Glycerol+ TAP), group III(Chitosan+ TAP), group IV(Clove oil+ TAP). Eight agar plates were taken, four plates were inoculated with E. faecalis and other four plates with C. albicans. Five wells were made in the agar with a metal tube to receive the testing material. The wells in each agar plate were filled with the intracanal medicament (TAP) and a particular vehicle. The zone of inhibition of microbial growth around the wells containing the medicaments was measured in millimetres after 24 hrs, day 3 and day 7. All the tests were done 3 times to minimize the test errors and for reproducibility of the results.

Results: Comparison of mean values were done using one- way ANOVA test. Intergroup comparisons were done with Post hoc Bonferroni test.

Intragroup comparison for Enterococcus faecalis and Candida albicans: In all the groups the mean zone of inhibition (ZOI) increased from day 1 to day 3 to day 7. For E. faecalis, the significant increase in ZOI is seen within the group I, II and IV from day 1 to day 7. In group III though mean ZOI is increasing from day1 to day 7 significant difference is not seen. For C. albicans, in group I and II though mean ZOI is increasing from day1 to day 7 significant difference is not seen. The significant increase in ZOI is seen within group III and IV from day 1 to day 7.

Intergroup comparison for Enterococcus faecalis: Group II (Glycerol+ TAP) has shown highest ZOI against E. faecalis compared to all other groups. After day 1 and day 3, there is significant difference in the groups Glycerol+ TAP and Clove Oil+ TAP with [GT> COT]; and also between Chitosan+ TAP and Clove Oil+ TAP with [CST> COT]. After day 7, there is significant difference between Glycerol+ TAP and control group [GT> ST]. Clove Oil+ TAP showed marked rise in ZOI after day 7 which is close to Glycerol+ TAP and Chitosan+ TAP.

Intergroup comparison for Candida albicans: Group II (Glycerol+ TAP) has shown highest ZOI against C. albicans compared to all other groups followed by clove oil and chitosan groups. After day 7, Group II, III and IV showed significant difference in ZOI compared to control group (Saline + TAP) with [GT> ST]; [COT >ST]; [CST> ST].

Conclusion: Among all the vehicles tested glycerol when used in combination with TAP showed highest zones of inhibition against both E. faecalis and C. albicans. Clove oil and chitosan also proved to be effective against both the organisms.

Keywords: Triple antibiotic paste, Enterococcus faecalis, Candida albicans, Chitosan, Clove oil, Glycerol.

Introduction

The success of endodontic treatment depends on effective debridement and disinfection of the root canal space. Failure to achieve a sterile root canal will result in residual infection or recontamination by microorganisms. Long-lasting endodontic infection might be attributed to the microorganisms in the entire root canal system including isthmi, ramifications, fins and dentinal tubules.[1] Studies have revealed that root filled teeth with persisting periapical lesions usually harbour various bacterial species & Gram-positive bacteria dominate the flora.[2] The organisms commonly found in cases of failed endodontic infections and endodontic flare-ups are E. faecalis and C. albicans.

Enterococcus faecalis has the ability to survive in root canal system as a single organism without the support of other bacteria and is small enough to proficiently invade upto1100µm within the dentinal tubules. It can be easily eliminated in planktonic forms in vitro but appears to be more resistant while it is present in an infected root canal system. E. Faecalis has potential role in contributing to endodontic failure due to certain factors such as its adherence to host cells, expressing proteins that allow it to compete with other bacterial cells, and altering host responses by activation of virulence factors, biofilm formation, and invasion of dentinal tubules.[4,5] While Enterococci have both intrinsic and acquired resistance to many antibiotics, they are inherently more resistant to antimicrobial drugs than other clinically significant Gram-positive bacteria.[3,6] C. albicans which is a fungi, usually cultured from root canals of teeth with failed endodontic treatment. Because of collagenolytic activity, it may be possible for the yeast to use dentin as a nutrient source and promote colonization in the root canal.[3]

Many studies have reported that E. faecalis and C. albicans are able to invade dentinal tubules to variable

depth. So, to ensure complete elimination of root canal bacteria, an effective antimicrobial agent in the root canal is required for a predetermined time period for complete eradication of any remaining bacteria. Calcium hydroxide has been widely used as an intracanal medicament in endodontics, but it has been shown to be ineffective at killing E. faecalis on its own, especially when a high pH is not maintained. E. faecalis resists calcium hydroxide as it passively maintains pH homeostasis by proton pump mechanism and buffering capacity of dentin will also lessen the effect of this intracanal medicament.[4,7] Swathi Pai et al in their study shown that E. faecalis and opportunistic infection causing microorganisms such as Candida, which are commonly found in diabetic patients, are found to be highly resistant to calcium hydroxide. [8]

Triple antibiotic paste (TAP) is a mixture of metronidazole, ciprofloxacin, and minocycline, has been widely used as an intracanal medicament for disinfecting the root canals during regenerative procedures. The infected root canal is not easily accessible for the local immune system and minimal concentration of the drug reaches the canal space after administration of systemic antibiotics which may not inhibit the bacterial growth adequately.[3] Therefore, the local application of antibiotics such as TAP within the root canal system may be a more effective mode of delivering the drug.

Drug delivery vehicles carry the drug to the target site inside any living system while increasing its stability and providing a sustained release of the drug. The antibiotic mixture has to be mixed with an appropriate vehicle for optimizing its intracanal placement. It helps the drug to penetrate through the infected dentin and eliminate the viable bacteria from the deeper recesses of the radicular dentin.[9] These vehicles are of three types such as aqueous vehicles (sterile water, sterile saline and anesthetic solution), viscous vehicles (glycerine, polyethylene glycol, propylene glycol) and oily vehicles (olive oil, camphorated paramonochlorophenol, metacresylacetate, eugenol).[10] Fava and Saunders in their study concluded that the vehicle with which calcium hydroxide medicament is delivered affects the physical and chemical properties of the compound and its clinical applications.[11,12]

In this study, four different vehicles were used to prepare the triple antibiotic paste, they are: Saline, Glycerol, Chitosan and Cloveoil.

Commonly called glycerol or glycerine, which is chemically represented as 1,2,3-propanetriol an important trihydroxy alcohol. Glycerol is found to exhibit certain antimicrobial properties against few bacteria, especially against Enterococcus species.[13]

Chitosan (CS) is a natural polysaccharide biopolymer usually obtained by alkaline partial deacetylation of chitin. Chitin is a straight homopolymer consisting of (1,4)-linked N-acetyl-glucosamine units, which can be found in the crustaceans exoskeletons such as crabs and shrimps. CS is generally regarded as biocompatible, non-toxic, and biodegradable and is inherently antibacterial in nature.[14] To increase the intracanal medicament stability, insoluble CS can be used as a drug carrier where it has added advantage of slow and controlled release of intracanal medicament.[3]

Oil of cloves, also known as clove oil, is an essential oil from the clove plant, Syzygium aromaticum. It main ingredient is eugenol because of which it has natural analgesic and antiseptic properties. The germicidal properties of the oil make it very effective for relieving toothache, sore gums, and mouth ulcers.[2] As a result, clove oil is added to numerous dental products and medications. Hence these three vehicles were used along with TAP and compared with saline for their antimicrobial efficacy.

The aim of this study is to analyse the sustained release of TAP using different vehicles and testing their antimicrobial efficacy against E. faecalis and C. albicans.

Methodology

Preparation of the medicament: A TAP was prepared by mixing 200 mg ciprofloxacin, 400 mg metronidazole, and 100 mg minocycline. It was prepared by removing the coating and crushing of antibiotic ciprofloxacin, metronidazole, and minocycline tablets separately using a mortar and pestle. The crushed powder was passed through a fine sieve to remove heavy filler particles and obtain a fine powder. The ciprofloxacin, metronidazole, and minocycline powders thus obtained were weighed separately and taken in 1:1:1 ratio. This mixture was dispensed and mixed with each vehicle medium (1:1) to get a thick paste-like consistency, and grouped according to vehicle used along with TAP as follows.

Group I: Saline+ TAP [ST] – control group

Group II: Glycerol+ TAP [GT]

Group III: Chitosan+ TAP [CST]

Group IV: Clove Oil+ TAP [COT]

Chitosan which is in powder form is treated with 1% acetic acid solution and the mixture was stirred in magnetic stirrer for 2 hours to obtain chitosan solution.

Screening of antibacterial activity (Agar well diffusion method): Eight petri dishes were taken and prepared with a lawn culture of the test organisms from the bacterial suspension made matching 0.5 McFarland's standard on the Muller Hinton agar media using sterile cotton swab and the plates were dried for 15 minutes. Four plates were inoculated with E. faecalis and other four plates with C. albicans. Five wells were made in the agar with a metal tube to receive the testing material. The wells in each agar plate were filled with the intracanal medicament and particular vehicle. The zone of inhibition of growth around the wells containing the medicaments was measured in millimetres after 24 hrs, day 3 and day 7 as shown in figures 1 and 2. All the tests were done 3 times to minimize the test errors. The results obtained were statistically analysed.

Results

All the analysis was done using SPSS version 25.0. A p-value of <0.05 was considered statistically significant. Comparisons of mean values were done using one-way ANOVA and post hoc bonferroni test as shown in table 1-4 and graphs 1,2.

Intragroup comparison for Enterococcus faecalis: In all the groups the mean zone of inhibition (ZOI) increased from day 1 to day 3 to day 7. For Enterococcus faecalis, the significant increase in ZOI is seen within group I, II, IV from day 1 to day 7. In group III though mean ZOI is increasing from day1 to day 7 significant difference is not seen.

Intergroup comparison for Enterococcus faecalis: After day 1 and day 3 analysis, the order of mean zone of inhibition is

(Glycerol+ TAP) > (Chitosan+ TAP) > (Saline + TAP) > (Clove Oil+ TAP)

After day 7 analysis, the order of mean zone of inhibition is

(Glycerol+ TAP) > (Clove Oil+ TAP) > (Chitosan+ TAP) > (Saline + TAP)

Group II (Glycerol+ TAP) has shown highest ZOI against E faecalis compared to all other groups. After day 1 and day 3, there is significant difference in the groups Glycerol+ TAP and Clove Oil+ TAP with [GT> COT]; and also between Chitosan+ TAP and Clove Oil+ TAP with [CST> COT]. After day 7, there is significant difference between Glycerol+ TAP and control group. Clove Oil+ TAP showed marked rise in ZOI after day 7 which is close to Glycerol+ TAP and Chitosan+ TAP.

Intragroup comparison for Candida albicans: In all the groups the mean zone of inhibition increased from day 1 to day 3 to day 7. In group I and II though mean ZOI is increasing from day1 to day 7 significant difference is not seen. The significant increase in ZOI is seen within group III and IV from day 1 to day 7.

Intergroup comparison for Candida albicans: After day 1 and day 3 analysis, the order of mean zone of inhibition is

(Glycerol+ TAP) > (Chitosan+ TAP)> (Clove Oil+ TAP) > (Saline + TAP)

After day 7 analysis, the order of mean zone of inhibition is

(Glycerol+ TAP) > (Clove Oil+ TAP) > (Chitosan+ TAP) > (Saline + TAP)

Group II (Glycerol+ TAP) has shown highest ZOI against C. albicans compared to all other groups followed by clove oil and chitosan groups. After day 7, Group II, III and IV showed significant difference in ZOI compared to control group (Saline + TAP) with [GT> ST]; [COT >ST]; [CST> ST].

Discussion

The complex nature of root canal system leave certain microbes untouched by the mechanical instrumentation and irrigation.[5] Intracanal medicaments plays pivotal role in elimination of bacteria from the root canals. In dentistry antibiotics are used both systemically and topically. During systemic administration of antibiotics, negligible concentrations reach the root canal space, whereas during the local administration of antibiotics, greater concentrations of the drug will be delivered to the required site decreasing systemic complications. Due to the diversity of root canal micrflora, single irrigant or a medicament or an antibiotic could not result in effective sterilization of the root canal. Hence a combination of antibiotics decreases the development of resistant bacterial strains and produces synergistic effect, whose antimicrobial action lasts longer and also sustained release of medicaments occurs.[3,15]

Grossman introduced first antibiotic paste called “PBSC” or “polyantibiotic paste” which is a mixture of penicillin, streptomycin, bacitracin and caprylate sodium. Penicillin was effective on gram-positive organisms, streptomycin for gram-negative organisms, bacitracin for penicillin-resistant strains and caprylate sodium to target yeasts.[16] TAP has been proposed as a root canal medicament due to its antimicrobial effects in endodontic procedures. It is effective for disinfection of the infected necrotic tooth, setting conditions for the subsequent revascularization of the tooth.[16] In an experimental study on dogs, the intracanal drug delivery of a 20 mg/ml solution of TAP resulted in >99% reduction in mean CFU levels.[17]

The use of three Mix-MP TAP was developed by Hoshino and colleagues. It consists of ciprofloxacin, metronidazole, and minocycline. Minocycline is in the bacteriostatic subgroup of antibiotics. This group of drugs are safe because when the bacterial cells are not lysed, there will not be any antigenic byproducts released in the infected area (such as endotoxins). Metronidazole is a nitroimidazole compound which is bactericidal and exhibits a broad spectrum of activities against protozoa and anaerobic bacteria. Ciprofloxacin is a second-generation fluoroquinolone antibiotic used for its broad spectrum of activity, their availability in both oral and intravenous formulations and their excellent tissue penetration.[18]

The use of local antibiotics becomes mandatory in endodontic cases as many non-vital and abscessed teeth lack blood circulation. As a result of this, systemic antibiotics fail to reach the site of infection and hence, such infections cannot be treated. Also, local drug delivery and sustained release along with better diffusion

into the surrounding periradicular tissues may prove to be an added advantage.[12] The vehicles used to dispense the intracanal medicaments have a direct influence on the release, time of onset of action of the medicament, penetration of the intracanal medicaments into dentinal tubules, and also the dissociation of drugs.[12]

In this study against E. faecalis and C. albicans, glycerol or glycerine proved to be the better vehicle as it exhibits antimicrobial activity by release of hydrogen peroxide. Another reason for its efficacy might be due to its high molecular weight that minimizes the dispersion of TAP into the dentin and due to its viscosity maintains the paste in the area for longer intervals leading to prolonged action of the paste.[8]

Chitosan has a number of important pharmaceutical applications. It has been used in drug delivery, as an absorption enhancer, colon targeting and gene delivery. There is no sufficient data available till date on the antimicrobial effect of TAP using chitosan as a carrier on C. albicans and E. faecalis.[3] Chitosan polymer is hydrophilic and adsorbed to canal wall which favours intimate contact. In addition, it has cationically charged amino group which combines with anionic components such as N-acetyl muramic acid, sialic acid, and neuramic acid on the cell surface and suppresses growth of bacteria by impairing the exchanges with medium, chelating transition metal ions, and inhibiting enzymes.[13]

In the present study, TAP + chitosan showed better antimicrobial efficacy against C. albicans and E. faecalis. The probable reason might be chitosan as a drug carrier has the advantage of slow and controlled drug release, which improves drug solubility, stability, enhancing efficacy, and reduced toxicity. The study by Aleksandra et al. also showed that chitosan has better antifungal activity against C. albicans.[19] The current results are in accordance with the study by Jaheer Shaik et al, who concluded that TAP + chitosan combination has shown better antibacterial and antifungal activity against C. albicans and E. faecalis when compared with TAP + saline combination.[3]

TAP + clove oil showed antimicrobial efficacy almost similar to glycerol and chitosan. Our results are in agreement with study by Madhavan. S et al who concluded that clove oil has enhanced antibacterial effect when combined with the other intracanal medicaments against E. faecalis.[2]

The lowest mean zone of inhibition for TAP + saline could be due to higher ionic dissociation caused by the lower viscosity of saline. Furthermore, the lower molecular weight of saline increases the dispersion of medication into the tissues minimizing its localization and prolonged action.[8]

Conclusion

Within the limitations of this study, it can be concluded that the type of vehicle utilized affects the diffusion ability and the antimicrobial activity of TAP. Among all the vehicles tested glycerol when used in combination with TAP showed highest zones of inhibition. Chitosan and clove oil also proved to be effective against both Enterococcus faecalis and Candida albicans. In vivo effects of these vehicles also need to be ascertained before their extrapolation to clinical conditions.

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Legend Figure and Tables

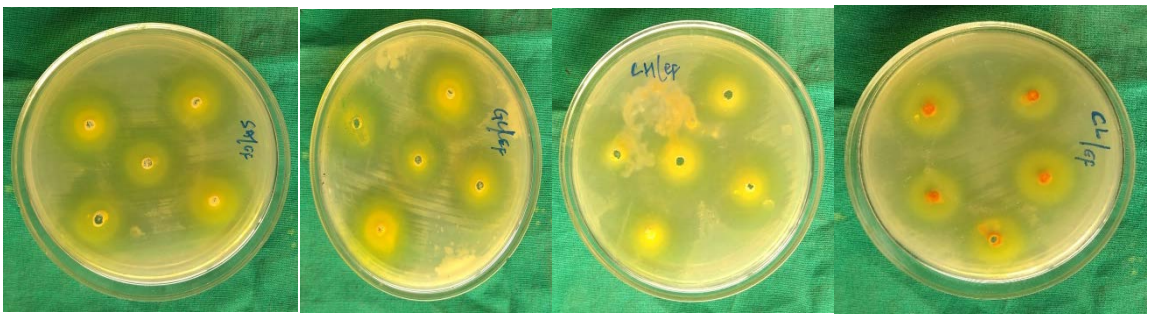


Fig 1: zone of inhibition of growth with medicaments for E. faecalis from group I to IV

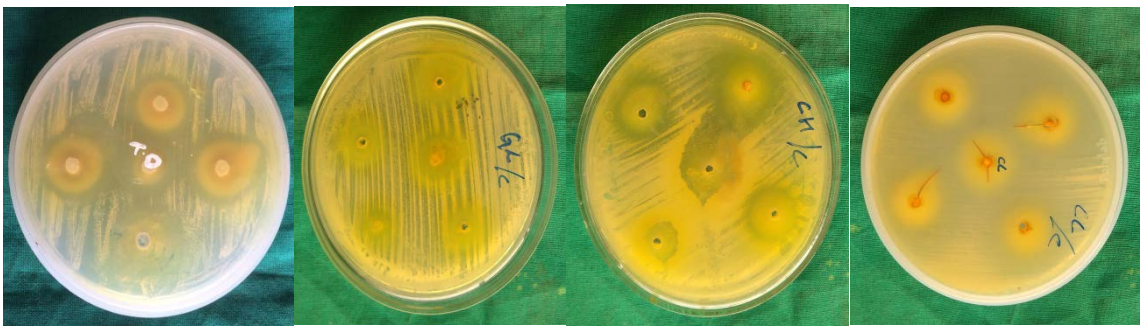


Fig 2: zone of inhibition of growth with medicaments for C. albicans from group I to IV

Table 1: Mean counts and standard deviation of CFU between four experimental groups for the organism Enterococcus faecalis (Intra Group)

Groups	Day1		Day3		Day7		P Value
	Mean	S.D	Mean	S.D	Mean	S.D	
Saline + Tap	16.3	0.57	18.1	0.74	18.3	0.4	0.000; Sig
Glycerol+ Tap	19.2	2.1	22.3	0.9	22.5	0.6	0.006; Sig
Chitosan+ Tap	19	2	20.1	0.89	20.5	1.1	0.243
Clove Oil + Tap	15.1	0.74	17.6	1.5	21	3.8	0.006; Sig

Table 2: Mean counts and standard deviation of CFU between four experimental groups for the organism Candida albicans (Intra Group)

Groups	Day1		Day3		Day7		P Value
	Mean	S.D	Mean	S.D	Mean	S.D	
Saline+ Tap	11.8	1.44	13.4	2.5	14.2	1.9	0.361
Glycerol + Tap	16.1	2.01	17.4	1.5	17.4	1.5	0.586
Chitosan+ Tap	13.9	0.74	15.3	0.67	15.3	0.67	0.01 ; Sig
Clove Oil + Tap	12.7	0.44	14.7	1.2	17.2	1.03	0.000; Sig

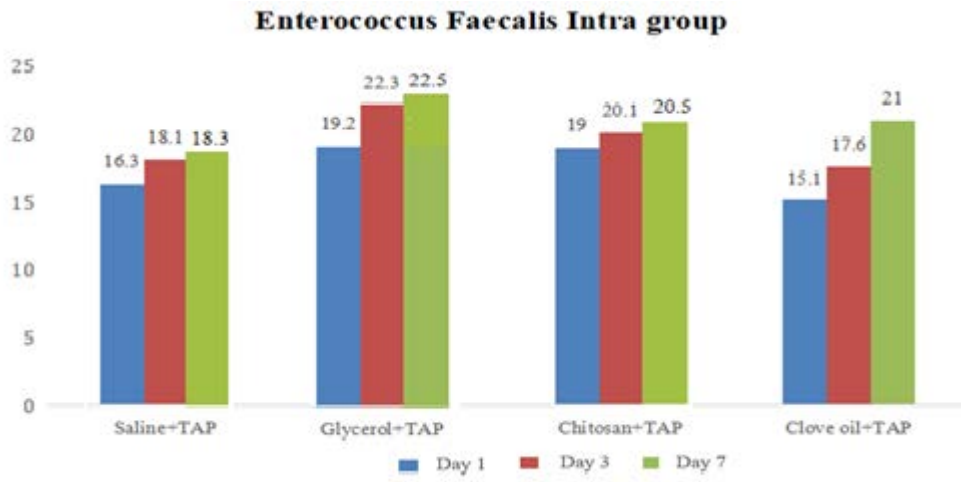
Table 3: Mean counts and standard deviation of CFU between four experimental groups for the organism Enterococcus faecalis (Inter Group)

Groups	Day 1		P Value	Day3		P Value	Day7		P Value
	Mean	S.D		Mean	S.D		Mean	S.D	
Saline + Tap	16.3	0.57	0.001; Sig	18.1	0.7	0.000; Sig	18.3	0.4	0.034; Sig
Glycerol + Tap	19.2	2.1		22.3	0.9		22.5	0.6	
Chitosan+ Tap	19	2		20.1	0.8		20.5	1.1	
Clove Oil + Tap	15.1	0.74		17.6	1.5		21	3.8	

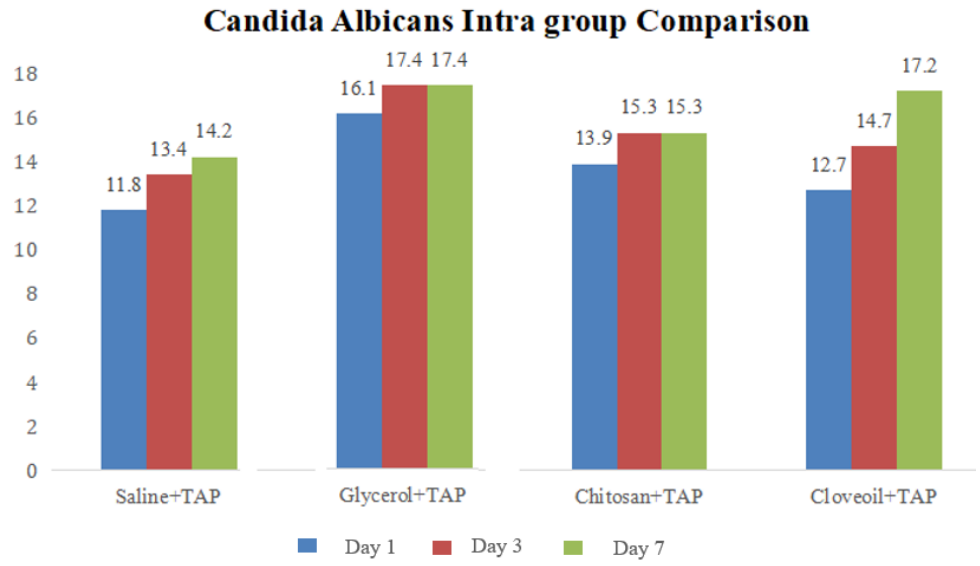
Table 4: Mean counts and standard deviation of CFU between four experimental groups for the organism Candida albicans (Inter Group)

Groups	Day 1		P Value	Day3		P Value	Day7		P Value
	Mean	S.D		Mean	S.D		Mean	S.D	
Saline + Tap	11.8	1.4	0.001; Sig	13.4	2.5	0.010; Sig	14.2	1.9	0.005; Sig
Glycerol + Tap	16.1	2		17.4	1.5		17.4	1.5	
Chitosan+ Tap	13.9	0.7		15.3	0.6		15.3	0.6	
Clove Oil + Tap	12.7	0.4		14.7	1.2		17.2	1.03	

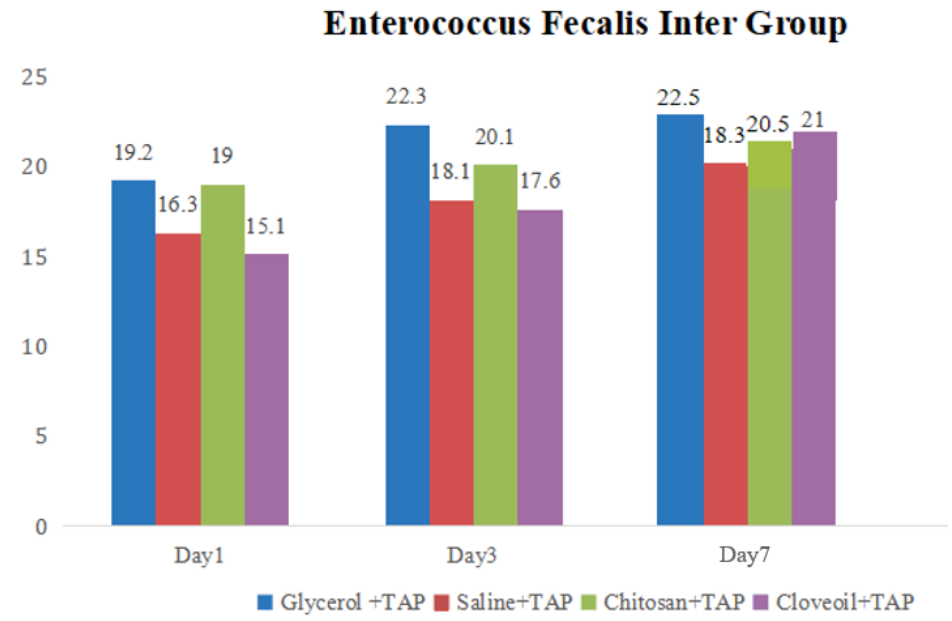
Graph 1



Graph 2



Graph 3



Graph 4