

 ISSN NO. 2320-5407	Journal Homepage: - www.journalijar.com INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR) Article DOI: 10.21474/IJAR01/10325 DOI URL: http://dx.doi.org/10.21474/IJAR01/10325	 INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR) ISSN 2320-5407 Journal Homepage: http://www.journalijar.com Journal DOI: 10.21474/IJAR01
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RESEARCH ARTICLE

COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFICACY OF TRIPLE ANTIBIOTIC PASTE AND MODIFIED DOUBLE ANTIBIOTIC PASTE USING DIFFERENT VEHICLES AGAINST CANDIDA ALBICANS

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Manuscript Info

Manuscript History

Received: 25 November 2019

Final Accepted: 27 December 2019

Published: January 2020

Key words:-

Triple Antibiotic Paste , Modified
Double Antibiotic Paste, Chitosan,
Chlorhexidine

Abstract

Endodontic infections are inherently polymicrobial in nature. These microorganisms flourish in harmonious consortium. No single microorganism can be held responsible for the failure of endodontic treatment or persistent apical periodontitis. The vivid flora involved in such incidences includes bacteria as well as fungi. Amongst the fungi *Candida* sp., *Aspergillus* sp, *Penicillium* sp. are the common ones to be isolated. Several studies have reported higher frequency of *Candida albicans* in cultures from root canals of teeth of failed endodontic treatment. Though chemomechanical preparation of root canal eliminates most of the microbes, complete sterility of root canals is difficult to achieve. Intracanal medicaments come into play in such situations. The triple antibiotic paste is most widely used intracanal medicament in present-day. This study evaluated the antimicrobial activity of triple antibiotic and modified double antibiotic paste with different vehicles against *C. albicans*. The antimicrobial efficacy was evaluated by using agar well diffusion method. Medicaments were placed in wells on agar plates previously inoculated with *C. albicans*. All plates were incubated and the diameter of bacterial inhibition zones on agar plate was measured in millimeters at 24, 48 and 72 hours at 37°C and 100% humidity. Result showed that Triple antibiotic paste was better than double antibiotic paste. TAP + CHX = TAP + chitosan > DAP + CHX > DAP + chitosan. Triple antibiotic paste (TAP) with chlorhexidine and TAP with chitosan had equal effectiveness, and Modified Double antibiotic paste (DAP) with chlorhexidine had better efficacy than DAP with chitosan.

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Introduction:-

Endodontic infections are characteristically polymicrobial in nature. These microorganisms flourish in harmonious consortium. No single microorganism can be held responsible for the failure of endodontic treatment or persistent apical periodontitis.

The vivid flora involved in such incidences includes bacteria as well as fungi. Amongst the fungi *Candida* sp., *Aspergillus* sp, *Penicillium* sp. are the common ones to be isolated. Several studies have reported higher frequency of *Candida albicans* in cultures from root canals of teeth of failed endodontic treatment. Though chemomechanical preparation of root canal eliminates most of the microbes, but complete sterility of root canals is difficult to achieve.

These microbes require antimicrobial agents. Systemic application of antimicrobial agents has its own adversities, the infected root canal is not accessible to the local immune system and hence the amount of drug reaching canal space is minimal and unlikely to inhibit the bacterial growth, this incompetence of systemic antibiotics in endodontic conditions has resulted in the use of local application of antimicrobial in root canal treatment. (1,2)

Intracanal medicaments come into play in such situations. Polyantibiotic paste containing penicillin, bacitracin, streptomycin and caprylate sodium is the first reported locally used antimicrobial intracanal medicament.(3) Considering the polymicrobial nature of endodontic infections, tetracyclines, a group of broad-spectrum antibiotics that are effective against a wide range of microorganisms, was proposed as intracanal topical antibiotics.

A conceptual swing has occurred from conventional to broad-spectrum treatment and now to combination modality due to vast variety of microorganisms encountered in the root canals. There have been studies on effectiveness of calcium hydroxide, chlorhexidine, fluconazole, chitosan, triple antibiotic paste etc. against *E. faecalis* and *C. albicans*.

Triple antibiotic paste is the most successfully and widely used intracanal medicament in present-day. Triple antibiotic paste (TAP), that was 1st used by Hoshino E et.al in 1996, is a mixture of metronidazole, ciprofloxacin, and minocycline. It has proven its effectiveness against *E. faecalis* and so is widely used. Since the endodontic infections are polymicrobial effectiveness of any intracanal medicament against single microorganisms does not makes it best choice

To increase the stability of intracanal medicament, chitosan is used as a drug carrier. It has drawn attention of dental researchers due to its biocompatibility, biodegradability, bioadhesion and lack of toxicity, it has additional advantage of slow and controlled release of intracanal medicament. (4,5,6) It is produced by the partial deacetylation of chitin which is a natural polysaccharide composed of β (1 $\rightarrow$ 4) linked N-acetyl glucosamine units. Chitosan derived from the exoskeleton of crustaceans (such as crabs) & is a cationic polymer. Its important property, to be used as excipient is because of its ability to become hydrated and form gels in acidic aqueous environments and is thus used to prepare slow release drug delivery systems.

Chlorhexidine (CHX) is known for its broad-spectrum antimicrobial and high substantivity. Efficacy of Chlorhexidine is because of the interaction of this cationic molecule with the anionic phosphate groups on the microbial cell wall. Which results in shifting of osmotic equilibrium of the cell and increased permeability of cell wall which allows the Chlorhexidine molecule to penetrate the bacteria.

Adding a vehicle to the intracanal medicaments not only improves the handling characteristics, but also improves antimicrobial activity, enhances diffusion through dentinal tubules and release of the medicaments. (6,7)

The present study was designed to evaluate the antimicrobial activity of triple antibiotic and modified double antibiotic paste with chlorhexidine and chitosan as vehicles against *C. albicans*.

Materials And Method:-

Preparation of medicaments:

1. Group A- Modified double antibiotic paste (8) : It was prepared by removing the coating and crushing of antibiotic Amoxicillin 500mg + Clavulanic acid 125 mg (Moxclav 625mg, Sun Pharmaceutical Industries Ltd., India) and Metronidazole (Metrogyl 400 mg, J. B. Chemicals and Pharmaceuticals Ltd., India) tablet in a mortar and pestle. The powder thus obtained were weighed separately and mixed in a ratio of 1:1 by weight to obtain modified double antibiotic paste.
2. Group B- Triple antibiotic paste: It was prepared by removing the coating and crushing of antibiotic ciprofloxacin (Ciplox 500 mg, Cipla, India), metronidazole (Metrogyl 400 mg, J. B. Chemicals and Pharmaceuticals Ltd., India), and minocycline (Minoz 100mg, Cipla, India) tablets using a mortar and pestle. The ciprofloxacin, metronidazole, and minocycline powders thus obtained were weighed separately and mixed in a 1:1:1 proportions, respectively, to obtain TA mixture.

The crushed powder was passed through a fine sieve to remove heavy filler particles and obtain a fine powder.

Preparation of the medium for *Candida albicans*:

C. albicans (ATCC 10231) strain was used in this study. Cultures were maintained on Sabouraud Dextrose Agar (SDA, Sigma-Aldrich, USA) slants in stock cultures. Stock cultures were subcultured onto freshly prepared SDA plates and incubated at 35°C for 48 hours. Growths from freshly subcultured isolates were suspended in 10ml of sterile saline to obtain a turbidity of 0.5 McFarland standard (equivalent to 1.5×10^8 CFU/ml). Aliquots of the suspension containing *C. albicans* were spread on 10 different (9) 140-mm diameter Petri dishes containing Mueller-Hinton Agar medium (Merck, Darmstadt, Germany). Excess inoculum was removed with a pipette and the inoculated plates were dried for 15 minutes at 37°C. Each plate was divided evenly into 4 sections. In each section of each plate, a well of dimension 5 mm in diameter and 4 mm in depth was created with a sterile stainless steel cylinder. Each medicament prepared was placed in one well each. (Figure 1) All plates were incubated at 37°C under aerobic conditions, and zones of growth inhibition were measured at 24, 48, 72 hours using a plastic ruler and was recorded for each material. All statistical analyses were performed with the SPSS (PC version 10 software, IBM, NY, USA) statistical software package.

Triple Antibiotic Paste : Mix Of Equal Weight (1:1:1) Of Ground Metronidazole , Ciprofloxacin And Minocycline:

1. Triple antibiotic paste with chlorhexidine in a ratio of 1.5:1 (wt/vol) to obtain a paste like consistency. (TAP CHX)
2. Triple antibiotic paste was mixed with chitosan in a ratio of 1.5:1 (wt/vol) to obtain a paste like consistency. (TAP CHITOSAN)

Modified Double Antibiotic Paste : Mix Of Equal Weight (1:1) Of Ground Metronidazole , Amoxicillin With Clavulanic Acid:

1. Modified double antibiotic paste with chlorhexidine in a ratio of 1.5:1 (wt/vol) to obtain a paste like consistency. (DAP CHX)
2. Modified double antibiotic paste was mixed with chitosan in a ratio of 1.5:1 (wt/vol) to obtain a paste like consistency. (DAP CHITOSAN)

Result:-**Table 1:-** The mean diameter of growth inhibition zones of *Candida albicans* found at different hours.

Group	C. Albicans	24 hours mean +SD	48 hours Mean +SD	72 hours Mean + SD
A1	TAP CHX	26.0 + 2.6	26.2+1.3	27.2+0.6
A2	TAP CHITOSAN	25.2+1.6	25.6+1.2	25.0+1.2
B1	DAP CHX	22.0+1.2	22.4+1.6	23.0+1.2
B2	DAP CHITOSAN	13.0+2.2	12.6+1.2	13.0+0.2

Overall, group A1 had the largest zones of growth inhibition at all time intervals when compared to other group.

Table 2:- Comparison of groups.

Comparison	T -score	p- value
TAP CHX vs TAP CHITOSAN	0.829	Not significant
TAP CHX vs DAP CHX	4.4	<0.05 significant
TAP CHX vs DAP CHITOSAN	12.14	<0.05 significant
TAP CHITOSAN vs DAP CHX	5.07	<0.05 significant
TAP CHITOSAN vs DAP CHITOSAN	14.18	<0.05 significant
DAP CHX vs DAP CHITOSAN	11.39	<0.05 significant

Group A1 also had significant inhibitory effects on *C. albicans* at all time intervals compared to other control group ($p < 0.05$). Intra group comparison of group A did not show significant difference whereas intragroup comparison of group B showed significant difference.

The Inter group comparison after 24 hours revealed statistically significant differences when Group A was compared with Group B ($p < 0.05$).

For Candida albicans:

1. Triple antibiotic paste(TAP) with chlorhexidine(CHX) and TAP with chitosan had equal effectiveness
2. Modified Double antibiotic paste (DAP) with CHX had better efficacy than with chitosan
3. TAP + CHX = TAP + chitosan > DAP + CHX > DAP + chitosan

Discussion:-

Both systemic and topical antibiotics are used in dentistry. Systemic administration of antibiotics allows only negligible concentrations of drugs to reach the root canal, whereas the local administration allows the greater concentrations of drug to be used as intracanal medicaments, additionally decreases systemic consequences and complications. Use of intracanal medicaments eliminates higher proportions of bacteria from the root canals.(10)

Complexity of root canal infections does not allow single irrigant or a medicament to be effective for sterilization of the root canal. Combination of medicaments decreases the chances of development of resistant bacterial strains and produces synergistic effect, and their antimicrobial action is long lasting with sustained medicaments release.(11)

In an earlier study by Chandra et al. found that Modified Double antibiotic paste irrespective of vehicle was more effective than triple antibiotic paste against *Enterococcus faecalis*. *C. albicans* being the next common culprit in cases of endodontic treatment failure was taken to carry out similar study.(12) The result of study by Shah et al. indicates the need for using intracanal medicaments and irrigants which have both antibacterial as well as antifungal action.(13)

Shaik et al. used TAP, Chitosan, Calcium hydroxide combinations against *C. albicans*. (14) In their study highest antifungal activity was seen with calcium hydroxide-chitosan and TAP-chitosan. Further more calcium hydroxide-chitosan has shown better antifungal efficacy against *C. albicans* compared with TAP- chitosan. Such result can be explained by synergistic/additive effect found in $\text{Ca}(\text{OH})_2$ combined with chitosan which possibly chitosan inhibit the growth of *C. albicans* and affects their re-entry and recolonization.(15)

R J Delgado et al. observed that CHX or its combination with $\text{Ca}(\text{OH})_2$ was effective antimicrobial when used as an intracanal medicament against *C. albicans*, even more effective than $\text{Ca}(\text{OH})_2$ alone.(16)

In 2 different studies Carbajal Mejía, J. B. et al. (17) and Chua, E.G. et al.(18) observed that CHX had the higher antifungal activity on *C. albicans* than propolis and triple antibiotic paste.

Our results indicate that all the medicaments resulted in decline of microbial growth which was evident after measuring zone of inhibition. Group A1 (TAP-CHX group) was significantly more effective at killing *C. albicans* than TAP-chitosan. Thus combining chitosan with TAP could not prove its efficacy over TAP CHX combination in eradicating *C. albicans*. Furthermore unlike against *E.faecalis*, (12) triple antibiotic remains superior choice against *C. albicans* over DAP.

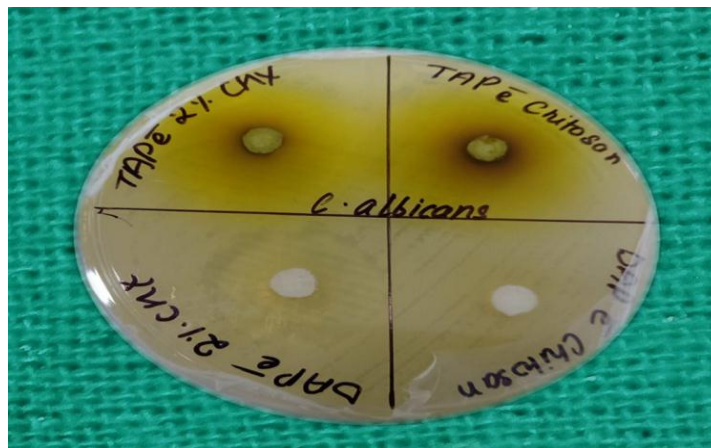


Figure 1:-MH agar culture plate after 72 hours at 37°C and 100% humidity.

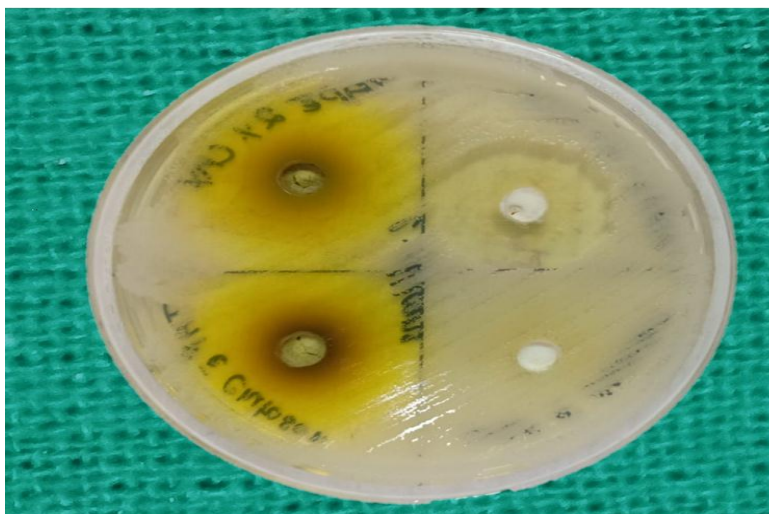


Figure 2:- Zone of inhibition seen on culture plate after 72 hours at 37°C and 100% humidity.

Conflict of interest:

Authors declare that there is no conflict of interest.

Conclusion:-

For obtaining maximum clinical benefits of the anti-microbial agents as intracanal medicaments, further research should be conducted to investigate the best drug delivery form, substantivity and the drug combinations. No single combination of drugs is effective against all microbes. Thus choosing intracanal medicament in each case is a serious task and should be cautiously carried out to obtain maximum benefit without giving rise to resistant strains.

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