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COMPARISON OF ANTI-BACTERIAL AND ANTI-INFLAMMATORY PROPERTIES OF NEEM, CURCUMIN AND ALOE VERA IN CONJUNCTION WITH CHLORHEXIDINE AS AN INTRACANAL MEDICAMENT – AN IN-VIVO STUDY.

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AIM & OBJECTIVE: The main aim of this study was to evaluate the potential of Neem, Curcumin and Aloe vera as an effective intracanal medicament in root canal therapy of infected teeth and to determine the antimicrobial and anti-inflammatory properties of these three herbal substances used in the study. **MATERIAL AND METHOD**: In this study,thirty three patients(33) within the age group of 25-40 years with two single rooted anterior teeth, presenting with periapical radiolucency requiring endodontic therapy were selected. These 33 patients were divided into two groups, in which one was the test and another was control in one single patient. The control samples were taken from all the 33 patients i.e (33 samples) in which chlorhexidine is used as intracanal medicament, whereas the test groups were divided into another three respective groups with 11 samples in each group on the basis of three herbal intracanal medicaments used in this study. The antimicrobial and anti-inflammatory property was assessed in our study using the microbial colony count method and the Visual analogue pain scale method respectively. **RESULTS:** Test Group A(Neem) showed the highest antibacterial activity with maximum reduction in microbial colony count scores. and the test Group B(Curcumin) showed the highest anti-inflammatory activity with maximum reduction in the VAS pain score. On comparing test groups with control group and antimicrobial property was analyzed. The results were statistically non-significant when Group A(Neem) was compared with Control group(Chlorhexidine) and statistically significant when Group B(Curcumin) and Group C(Aloe vera) was compared with Chlorhexidine.

Key words: Aloe vera, Curcumin, Chlorhexidine, Neem, Peptone Agar, Robertsons Cooked Meat Media

INTRODUCTION

Dental caries is a localized destructive and progressive infection of dentin, if left untreated results in pulpal necrosis. Both bacterial by products and products from the dissolution of the organic and inorganic constituents of dentin mediates the effects of dental caries and the pulp which leads to pulpitis.[1] Pulpitis or inflammation of pulp can be reversible or irreversible. Irreversible pulpitis is a persistent inflammatory condition of the pulp caused by a long-standing noxious stimulus such as caries, which penetrated the dentin, causing inflammatory changes in the pulp and progresses to necrosis and causing root canal treatment.[2] The goal of endodontic treatment is to remove and kill all micro-organisms in the root canal. Endodontic therapy involves the removal of infected tissues and microorganisms from within the root canal space to prevent further infection of the periradicular tissues, so that it can be sealed with a microbial-tight filling. This process mainly involves the use of certain medicaments for disinfection of the root canal space and is known as "chemo-mechanical preparation.[3]"

Nature Helps....offcourse!!

One of the most versatile, benign and perhaps the most useful traditional medicine given by nature and used in our study is Neem- A Green Treasure.

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Another medicinal plant which is extensively used and is a popular Indian spice is Turmeric, otherwise called as Curcuma Longa.

Another herbal medicine, widely popular now a days, is a kind of herbal medicine that has been widely used in basic health care in many countries is the Aloe vera.[4]

2% Chlorhexidine gel is a widely used and accepted intracanal medicament and irrigant used in endodontics. This present study was conducted as our effort to further progress the research work in the field of herbal medicine so as to use the blessings of mother nature in the service of mankind , especially so in endodontics.

MATERIAL & METHODOLOGY

Materials used in this in-vivo study

1. Azadirachta Indica Extract powder
2. Curcumin powder
3. Aloe Vera Extract powder
4. Chlorhexidine Solution (Indico Remedies)
5. Intermediate Restorative Material (Dentsply Maillefer, Switzerland)
6. 17% EDTA solution (Prime Dental Products, India)
7. Zinc Oxide powder (Deepak enterprise, India)
8. Eugenol oil (Givaudan)
9. Gutta percha points (Sure Endo, Korea)
10. Peptone agar (Himedia, India)
11. Robertson's Cooked Meat Media (Himedia, India)
12. Blood agar plates (Himedia, India)
13. Anaerobic Gaspak (Himedia, India)

Grouping

In this study, 33 patients, with two teeth presenting with periapical radiolucency were divided into two groups, in which one was the test and another was control, in one single patient.

The control samples were taken from all the 33 patients i.e (33 samples) in which chlorhexidine is used as intracanal medicament, whereas the test groups were divided into another three respective groups with 11 samples in each group on the basis of three herbal intracanal medicaments used in this study, therefore, in total sixty six samples (66) were taken .

Group A: Neem was used as a test intracanal medicament.

Group B: Curcumin was used as a test intracanal medicament.

Group C: Aloe Vera was used as a test intracanal medicament.

Chlorhexidine was used as a control intracanal medicament in all the three groups (Figure 1)

Figure 1: All the samples were included in the study

The antimicrobial and anti-inflammatory property was assessed in our study.

The antimicrobial property was analyzed using the microbial colony count method by counting the number of microbial scores.

As anti-inflammatory property of intracanal medicaments was assessed, which was done on the basis of measuring the pain intensity by using the Visual Analogue Pain Scale.(Figure 2)

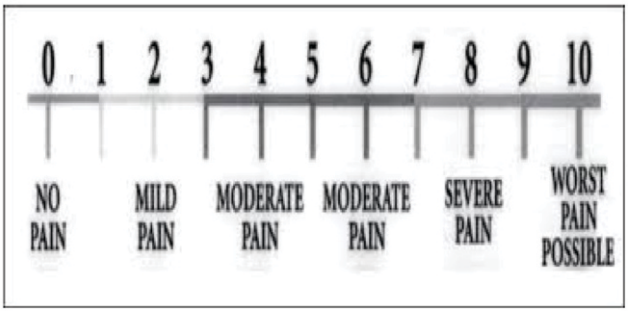

Figure 2: Visual Analogue Pain Scale

Three herbal intracanal medicaments, Neem, Curcumin and Aloe vera were used as an intracanal medicament in our present study were prepared in 50% conc by mixing 10 gms of Curcumin , Neem and Aloe vera in 10 ml of DMSO(Dimethylsulfoxide) and Distilled water respectively in a petridish and mixing it continuously with a stirrer, to obtain a flowable

consistency (Figure 3).

Figure 3

PROCEDURE

Collection Of Initial Samples

For analyzing the antimicrobial assessment of the three intracanal medicaments, the procedure has been followed. After administration of local anaesthesia, rubber dam isolation was done. The access to the pulp chamber was obtained and the canals were identified using a 10-K hand file and the working length was determined by reducing 1mm from this length. H-file was used to remove the debris from the canal walls. The exudate from the canal was collected by inserting two paper points in the canal two times, one after the other upto the noted length. Debris and exudates was collected for both aerobic and anaerobic cultures.

The initial samples for aerobic culture was collected in Peptone water and samples for anaerobic culture were collected in Robertson's Cooked Meat Media. The collected samples were incubated at 37°C for 48 hours in an incubator. After 48 hours samples from aerobic and anaerobic media were taken and two separate blood agar plates were inoculated for microbial colony count by Streak culture (Surface plating) and the growth was checked at the site of original inoculation (Well).

The anerobic culture plates were maintained by keeping the inoculated culture plate in the Gaspak which is a simple and effective method of choice for preparing anaerobic jars.

Collection of Final Samples

After the working length was determined the biomechanical preparation of the teeth was done in the first appointment itself using Step-back method. Copious irrigation with normal saline was done during the preparation.

For Group A, Neem paste was introduced in the canal coated on two paper point and the coronal access was sealed with an intermediate restoration and similarly in another tooth, 2% Chlorhexidine immersed paper point as (Control) was introduced into the canal, as an intracanal medicament and the canal orifice was sealed using Intermediate restorative material in another tooth.

On recall after 2 days pain was analyzed by the pain assessment scale (VAS) for assessing the anti-inflammatory effect of the medicaments used. The tooth inoculated with Azadirachta (Neem) was isolated again. The canal orifice was again opened and the azadirachta sample (test) and chlorhexidine sample (control) was removed for aerobic and anaerobic culture. Similar regimen of treatment was followed for Group B (Curcumin) and Group C (Aloe vera).

Comparison of Samples

The comparison between the initial (pre medication) and final (post medication) microbial colony count was done to determine the antimicrobial effect of the medication. The visual analogue scale score before and after instrumentation was calculated to determine the anti-inflammatory property of the medicament.

RESULTS

On comparing the three test groups, Group A(Neem), Group B(Curcumin) and Group C(Aloe vera) and assessing the antimicrobial properties, Group A (Neem) showed the maximum antimicrobial potential and offered better results which is statistically significant as compared to the other two Groups B (Curcumin) and Group C(Aloe vera).

The anti-inflammatory property was seen maximum in Group B(Curcumin) which is statistically significant with maximum reduction in the VAS Pain score when compared to Group A(Neem) and Group C (Aloe vera)

When the three Test groups, Group A (Neem), Group B (Curcumin) and Group C (Aloe vera) were compared with the Control group (Chlorhexidine) and anti-inflammatory property was assessed post-medication, The Test groups (Neem, Curcumin and Aloe vera) showed better results with maximum reduction in the VAS pain score. (values of Table 1 based on Non Parametric Test)

Table 1: Descriptive Statistics (Non Parametric test)

GROUP		N	Mean	Std. Deviation	Minimum	Maximum	Percentiles		
							25th	50th (Median)	75th
NEEM	Vas Pain Scores Pre-medication	11	6.727	1.1037	5.5	8.5	6.000	6.000	8.000
	Vas Pain Scores Post-medication	11	2.545	.9863	1.0	4.5	2.000	2.500	3.500
CHLORHEXIDINE	Vas Pain Scores Pre CONTROL	11	7.1818	1.07872	6.00	9.00	6.5000	7.0000	8.0000
	Vas Pain Scores Post CONTROL	11	3.5455	.78913	2.50	4.50	3.0000	3.5000	4.5000
CURCUMIN	Vas Pain Scores Pre-medication	11	5.955	1.1501	4.5	8.0	4.500	6.000	7.000
	Vas Pain Scores Post-medication	11	1.591	.7687	.5	2.5	1.000	1.500	2.500
CHLORHEXIDINE	Vas Pain Scores Pre CONTROL	11	6.6818	1.58545	4.50	9.00	5.0000	6.5000	8.0000
	Vas Pain Scores Post CONTROL	11	2.8182	.90202	1.50	4.00	2.0000	3.0000	3.5000
ALOEVERA	Vas pain scores Pre-medication	11	6.818	1.1017	5.0	9.0	6.000	7.000	7.500
	Vas Pain Scores Post-medication	11	3.364	.8970	2.0	5.0	2.500	3.500	4.000
CHLORHEXIDINE	Vas Pain Scores Pre CONTROL	11	6.7727	1.21169	5.00	9.00	5.5000	7.0000	7.5000
	Vas Pain Scores Post CONTROL	11	3.8636	1.12006	2.50	5.50	2.5000	4.0000	5.0000

When the three test groups were compared with control group and antimicrobial property was assessed. The results were statistically non-significant, when Group A(Neem) was compared with Control group (Chlorhexidine) and statistically significant when Group B(Curcumin) and Group C (Aloe vera) was compared with control group Chlorhexidine. (values of Table 2 based on t-Test)

DISCUSSION

Caries can be compared with a train that passes through many stations. Imagine that each station represents a specific stage of caries progression. The first station represents the initial surface etching at the outer enamel layer, leading to the dull white appearance of the active progressing enamel lesion. The last station represents the deepest layer of the carious tooth, with a necrotic, infected root canal system and the presence of apical pathosis.[5]

Pulpal or periradicular inflammation results from irritation or injury usually from bacterial source. Bacteria usually from dental caries, is the main source of injury, and they either enter directly or through dentinal tubules. The response of the pulp depends on the severity of the insult and may result in a transient (reversible) inflammatory response or an irreversible one, which may eventually proceed to pulp necrosis. Irreversible pulpitis is a permanent nerve damage,

Table 2: t-TEST

GROUP			Mean	N	Std. Deviation	Std. Error Mean
Neem	Pair 1	Microbial Colony Count Pre	11.55	11	2.841	.857
		Microbial Colony Count Pre Control	12.1818	11	2.92637	.88233
	Pair 2	Microbial Colony Count Post	2.82	11	1.168	.352
		Microbial Colony Count Post Control	3.3636	11	1.28629	.38783
Curcumin	Pair 1	Microbial Colony Count Pre	12.55	11	3.078	.928
		Microbial Colony Count Pre Control	11.9091	11	2.16585	.65303
	Pair 2	Microbial Colony Count Post	5.00	11	1.949	.588
		Microbial Colony Count Post Control	3.7273	11	1.27208	.38355
Aloe Vera	Pair 1	Microbial Colony Count Pre	11.91	11	3.390	1.022
		Microbial Colony Count Pre Control	12.1818	11	3.12468	.94213
	Pair 2	Microbial Colony Count Post	5.27	11	1.618	.488
		Microbial Colony Count Post Control	4.4545	11	1.57249	.47412

usually occurs as a result of more severe insults than in the reversible pulpitis.[6]

Although chemo-mechanical preparation has an important cleaning effect, it cannot eliminate all the bacteria from the root canal system. The remaining bacteria may multiply during the period between appointments, often reaching the same level that it was at the start of the previous sessions, in cases where the canal is not dressed with a disinfectant between visits. This calls for the use of an effective intracanal medication that will assist disinfection of the root canal system. Such a medication should be effective throughout its period of application and penetrate the dentinal tubules, eliminating bacteria that may be present, with little toxicity to the periradicular tissues.[7] Owing to the potential side effects, safety concerns, ineffectiveness of conventional allopathic formulations and constant increase in antibiotic resistant strains, consumption of preparations from medicinal plants has increased over the last few decades.

Neem (Azadirachta Indica) used as a medicament in this study is a member of mahagony family is a traditional medicine well known in India and its neighboring countries. It is advantageous because it is biocompatible, antioxidant and thus not likely to cause the severe injuries to patients that might occur via sodium hypochlorite accidents.[8]

Curcumin : A Natural Antiinflammatory agent another herbal products which was used in our present study. It is a yellow coloured phenolic pigment, obtained from powdered rhizome of C.Longa Linn and is a member of the ginger family, Zingaberaceae.[9]

Whole leaf Aloe vera in dentistry is another such extract used as a medicament in our study. It is a potent anti-inflammatory agent and exhibits potent anti viral and anti tumor activity. It possess good anti bacterial and anti fungal activity. Showed significant Zone of inhibition against *E.faecalis* hence, it has shown antimicrobial effects against resistant microorganisms found in pulp space.[4]

2% Chlorhexidine is also used in our study.[10] It is a synthetic cationic bis-guanide that consists of two symmetric 4-chlorophenyl rings and two biguanide groups connected by a central hexamethylene chain. Aside from having exceptional antimicrobial activity, another favourable characteristic of chlorhexidine is substantivity. This property have led to the possibility that chlorhexidine is used as an effective intracanal medicament.[11]

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In this in-vivo study, two parameters were assessed, the antimicrobial action which was measured using the microbial colony count and the second is the anti-inflammatory action which was analyzed using the VAS pain score.

This VAS is a diagnostic tool that is used in assessing the severity and quality of pain experienced by the patient. The most common form used for pain is a 10 cm line, whether horizontal or vertical, with perpendicular stops at the ends. The ends are anchored by "No Pain" and "Worst Pain imaginable" (Figure 1)

The anti-bacterial property was analyzed by microbial colony count in which bacteria have to be cultured in the laboratory on artificial culture media.

(Neem) had the highest antibacterial activity and shows the least colony count and which is statistically significant when compared to other test Group B (Curcumin) and least in Group C (aloe vera). The antibacterial activity of Neem might be due to presence of triterpenoids, phenolic compounds, carotenoids, steroids, valavinoids, ketones and tetra-triterpenoids azadirachtin[12] (Figure 5).

Group B (Curcumin) showed the maximum anti-inflammatory activity with maximum reduction in the pain score which is statistically significant when compared to other test groups A (Neem) and (Aloe vera). Its anti-inflammatory activity is mainly due to inhibition of AA metabolism, COX, LOX, cytokines (ILs and TNF) and NF-κB. Curcumin inhibited NF-κB activation by blocking phosphorylation of I-B through inactivation of I-κB kinase complex[13] (Figure 6)

When all the three test Groups A,(Neem) B (Curcumin) and C(Aloe vera) were compared with the Control group (Chlorhexidine) post-medication for analyzing the anti-bacterial property using the microbial colony count. The results of the study concluded that Group A (Neem) and Control group (Chlorhexidine) had almost same colony count which was statistically non-significant , therefore possessing the maximum anti-bacterial property. (Table 2)

The results of Group B(Curcumin) and Group C(Aloe vera) were significant with the control group(Chlorhexidine). The anti-microbial effect is related to the cationic molecule binding to negatively charged bacterial cell walls, thereby altering bacterial osmotic equilibrium (Figure 7 and Figure 8).

When analyzing the anti-inflammatory property, the three test groups *Neem, Curcumin and Aloe vera* possess more anti-inflammatory property which is statistically significant with more reduction in the VAS Pain score as compared to Chlorhexidine. Out of which, Curcumin shows the best results. (Table 1)

Figure 5: Final culture plate post-medication for Neem

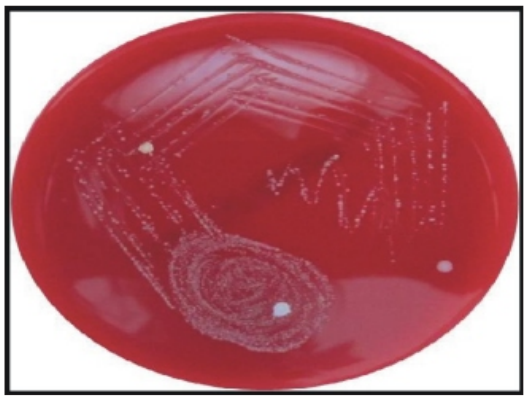

Figure 4: Culture plate pre-medication

Figure 6: Final culture plate post-medication for Curcumin

Figure 7: Final culture plate post-medication for Chlorhexidine

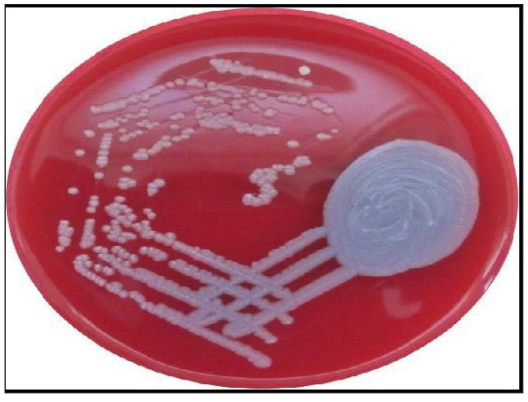

Figure 8: Final culture plate post-medication for Aloe vera

CONCLUSION

Oral diseases impact our quality of life and may lead to systemic and life threatening diseases Root canal infections are polymicrobial infections characterized by mostly anaerobic bacteria and some facultative bacteria. The spread of infection and the inflammatory response will continue until the source of the irritation is removed[14].

Although chemomechanical preparation has an important cleaning effect, it cannot eliminate all the bacteria from the root canal system because of various drawbacks of these conventional medicaments, there is a need for alternative therapies and herbals make a good alternative.[15]

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